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Chromosomes and Plant Cell Division in Space

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OBJECTIVES

A. EXAMINATION OF CHROMOSOMAL ABERRATIONS:

1. Assess and compare the frequency of chromosomal aberrations in plant material under space- and land-based conditions.

B. DEVELOPMENT OF EXPERIMENTAL SYSTEM:

1. Assess the suitability of two floral foam types for daylily and Haplopappus root growth studies employing tissue culture-derived plantlets.

Evaluation Criteria:

- (a) Successful procurement of newly generated root tips (indicative of a sufficient media-supplying capacity and a lack of toxicity attributable to the foam type and associated chemicals/wetting agents).
 - (b) Media holding capacity.
 - (c) Material characteristics relative to preparation and manipulation requirements.
2. Assess the suitability of seven nitex mesh sizes for daylily and Haplopappus root growth studies employing tissue culture-derived plantlets.

Evaluation Criteria:

- (a) Successful procurement of newly generated root tips (indicative of successful transport of media from the foam block matrix and a lack of toxicity attributable to the nitex).
- (b) Success in preventing significant root/root-hair penetration into the foam block matrix. It is essential that the root tips be removable without damage. The first level of damage would result from root attachment to the foam block matrix. Damage could also result from root entanglement within the nitex itself.

C. EDU EVALUATION:

1. Evaluate the performance of the PGC-EDU-AES system in terms of its being able to sustain sufficient plant growth conditions for shuttle-based investigations.

PLANT STUDIES

The primary objective of this year's efforts was to develop procedures for shuttle-based investigations which will result in the procurement of plant root tips for subsequent cytological examination. Two types of floral foam and seven nitex mesh sizes have been extensively tested for suitability in this endeavor. Initial investigations explored the overall characteristics of the foam substrata, including the manner in which liquids become distributed once added to the foam matrix.

Techniques employed in laboratory-based investigations were subsequently adapted for experiments simulating flight conditions employing the Engineering Design Units (EDUs) based at Stony Brook and at the Kennedy Space Center (KSC). The EDUs are experimental versions of the Plant Growth Unit (PGU) which will eventually fly in the shuttle.

Each EDU contains five Plant Growth Chambers (PGCs) which are stocked with the experimental organisms. Air flow will be provided to four of the five PGCs by the Air Exchange System (AES).

A. SPECIES SELECTION/JUSTIFICATION

A primary objective of this mission is to determine whether the "outer space" environment results in higher rates of chromosomal aberrations than normal background rates on earth. We are currently favoring the option of deploying both daylily (Hemerocallis cv. 'Autumn Blaze,' and Haplopappus gracilis plantlets within the PGCs. These plants represent both the dicotyledonous and monocotyledonous subclasses of higher plants. The following considerations have led us to their selection.

Several strains of daylily have been maintained under various tissue culture regimes in this laboratory for over ten years. The daylily constitutes one of the few model systems capable of going from cell suspensions to fully differentiated plants capable of survival in the field. As such, it provides an excellent choice for basic investigations on plant tissue culture manipulations in space.

With regard to Haplopappus gracilis, over the past year we have established capitulum-derived tissue cultures which have given rise to shoots, roots and flowers. Tissue cultures have also been initiated from newly germinated seedlings, and we have worked extensively with apex-derived tissue culture material (strain KH-1) cordially supplied by Dr. R. Tanaka, Laboratory of Plant Chromosome and Gene Stock, Hiroshima University.

The type of information required in the search for causes depends upon the types of factors which can cause chromosomal aberrations. Given that with tissue culture methodologies we can begin with material which is, for the most part, genetically uniform, the sources of genetic variation arise during the transmission of the genetic

information from cell to cell. Recombination can be dismissed since meiotic (reduction) divisions are not envisioned for these investigations. We are left with two primary candidates in the search for causes; (1) gamma rays acting as mutagenic agents, and (2) gravity-related phenomena which result in the disruption of the mitotic process.

The daylily and Haplopappus systems differ in several key respects. Haplopappus gracilis has the fewest number of chromosomes for any higher plant ($2n = 4$). Diploid cells contain about 4 pg DNA (Bennett and Smith, 1976). In contrast, the daylily (Hemerocallis cv. 'Autumn Blaze') diploid cell contains 22 chromosomes and most likely about ten times the quantity of DNA found in Haplopappus. Between them they represent plants containing a normal amount of genetic material and plants possessing a small amount of genetic material.

This quantitative difference in DNA per cell becomes important if gamma rays are a major cause of chromosomal aberrations. The principles of target theory could be applied. Plants containing relatively small amounts of DNA have less genetic material capable of interacting with gamma rays. The prediction would therefore be that Haplopappus should exhibit a reduced rate of abnormalities in comparison with the daylily.

Similarly, the comparison of species with different chromosome numbers could be enlightening if, for instance, a major cause of space-based chromosomal aberrations turns out to be disruption of the spindle apparatus during mitosis. Again, predictions can be made concerning the rate of abnormalities when but four chromosomes need be guided through cell division, in contrast to 22 chromosomes requiring error-free guidance in Hemerocallis sp.

B. EXPERIMENTAL SYSTEM DEVELOPMENTAL STUDIES

1. FLORAL FOAM - PH INVESTIGATIONS

Preliminary investigations were conducted on the pH altering characteristics of four foam substrata candidates obtained from Smithers-Oasis, 919 Marvin Ave., Kent, Ohio 44240. Types one and two were floral foam standard (FFS) and floral foam instant (FFI) formulations (both green). The latter is created by the additional treatment of creating 1-2 mm diameter holes throughout the standard foam in order to facilitate quicker saturation.

Of the two remaining foam types, the larger porosity foam (possessing a lower liquid holding capacity but a more rapid uptake potential) was brown Oasis floral foam type LC-1. The smaller porosity foam (with a larger liquid holding capacity but requiring longer periods to become saturated) was green Oasis floral foam root (FFRC) cubes (batch R-217).

Rectangular blocks (10.8 cm X 6.9 cm X 3.0 cm) were cut from the four foam types (each 4-4.5 gm. dry weight). They were inserted into

600 ml beakers containing distilled water (possessing an initial pH of ca 5.7). Determinations of pH were made over the next five days. During this interval, the FFS and FFI foam types depressed the distilled water pH values to 2.95 and 3.00 respectively. In contrast, foam types LC-1 and FFRC resulted in elevated pH levels (7.12 and 6.66 respectively).

Based upon these results, both the FFS and FFI foam types have been eliminated from further consideration. While their pH characteristics may result in prolonged maintenance of cut flowers, they were not deemed suitable for root formation. Subsequent studies concentrated on the evaluation of the FFRC and LC-1 formulations. Hereafter, we shall refer to the FFRC formulation as the "green foam" and the LC-1 formulation as the "brown foam."

2. MAJENTA-BASED STUDIES

We have routinely used what are referred to as "magenta" culture vessels for laboratory investigations (obtainable from the Magenta Corporation, Laboratory Products Division, 4149 W. Montrose Ave., Chicago, IL 60641). These rectangular containers (9.7 cm high X 6.6 cm wide X 6.6 cm long) are constructed of polycarbonate with a polypropylene top. Their transparency, and capacity for withstanding the rigors of multiple autoclavings have made them extremely convenient for plant tissue culture studies.

a. DAYLILY FOAM/NITEX EVALUATION STUDY

The following investigation evaluated the feasibility of generating Daylily root tips in the green (FFRC) and brown (LC-1) foam substrata types. Different sized nitex liner strips were also evaluated. These nitex liners effectively surround the root masses and are employed to prevent roots from penetrating into the foam matrix, a process which would result in damage to the root tips upon plant removal.

METHODOLOGY

The approach taken was to employ majenta culture vessels containing four different shaped blocks of both floral foam types. As such, the majentas approximated small plant growth chamber (PGC) units with the exception of there not being any air flow through the system. At this level, we are dealing with batch culture systems which are, for the most part, closed off from the ambient environment. In reality, there is low level gas exchange (estimated at 1-2 turnovers per day), but certainly nothing approaching the level of air exchange possible with the air exchange system (AES), which will be forcing air through four of the five PGCs contained within the PGU/EDU enclosures.

In all cases, block heights were intended to be relatively uniform at ca 3.2 cm (the range of block heights was 2.90-3.65 cm). This height was chosen for several reasons. First, it approximates

the level to which agar is usually added to the majenta culture vessels in more traditional plantlet grow-out studies (as performed in this laboratory for years). Second, this constitutes the greatest height with which we are comfortable given the initial and final sizes attained by experimental plantlets. Care must be taken not to overly restrict plant growth due to vessel size restrictions. In addition, the taller the blocks the more accessible the receiving slits when plantlets are inserted and/or removed. And finally, the taller the blocks the more media can be accommodated given any desired block shape.

Silicone was used for all small and medium sized foam blocks to localize them in the middle of the majenta culture vessels. The procedure was to place a "dab" of silicone on the center of the block bottom, invert the container, and press the foam block bottom up against the center of the majenta bottom, effectively spreading it out into a thin film covering an area of 2-4 cm² between the block and container.

This attachment treatment was not employed for the large and extra large size class blocks since their larger dimensions resulted in a snug fit against either all four sides of the majenta (for the extra large blocks) or in the case of the large sized blocks, against two opposite sides of the majenta.

The experimental plantlets were all procured from a single batch (Ab C-3 7/14) of daylily plantlets which had been growing within majentas on agar supplied with half strength Schenk and Hildenbrandt medium supplemented with 1% sucrose. Plantlets from several majentas were employed but they were all from the same batch and had experienced the same culture history.

The experimental design was as follows. There were eight small green and eight small brown foam blocks. Each had a ca 1 cm slit extending from the block's topmost surface down 2 cm. Each slit was lined with a nitex strip, effectively foaming a barrier between the plant and the foam. Seven nitex mesh sizes were tested (the smallest being 1, the largest 7). The mesh openings for nitex sizes 1-7 were 35 um, 46 um, 75 um, 103 um, 140 um, 190 um, 220 um and 590 um respectively. The eighth green and brown blocks were water loss controls, lined with nitex size 4 but not stocked with any plants during the study. Similarly, there were eight medium green and eight medium brown blocks, and eight large green and eight large brown blocks. For the extra large sized blocks, only three nitex mesh sizes were tested (1, 4, 7). As a result there were four blocks of the extra large green and brown foam types (each class including one control block with nitex mesh 4).

The experimental protocol was to aseptically remove the plantlets, trim the leaf bunches to 4 cm in length and the root complexes to ca 1/2 cm in length. The plantlets were then inserted into the foam blocks employing a pair of forceps, one to separate the nitex lining and widen the slit at the point of insertion, and the other to physically hold and insert the plantlet.

A preliminary study was performed to assess the amount of medium to be added to the blocks. The smallest blocks of each size class (for both foam types) were selected for these determinations so that the amount added would represent the minimum capable of being held by the different block-type-size combinations without drainage out of the blocks onto the majenta bottoms. Based on these efforts, it was determined that the small blocks could hold 12 ml and 9 ml for the green and brown foam types respectively. The medium sized blocks held 27 ml (green) and 17 ml (brown), the large held 72 ml (green) and 45 ml (brown), and the extra large 90 ml (green) and 70 ml (brown).

Prior to stocking the foam blocks with the experimental plantlets, premeasured amounts of one quarter strength Schenk and Hildenbrandt medium supplemented with 0.5% sucrose were added to small flasks (made up the day before, one flask for each block), and autoclaved. Media addition therefore merely required pouring the appropriate flask contents onto each foam block. Due to the larger block sizes (relative to those used for the estimate of block media holding capacities) and the inability to add the media evenly, dry spots were evident on all blocks. Consequently, the decision was made to add four more milliliters (one along each of the four topmost edges) to the following block treatments: Green Extra Large, Green Large and Brown Large. The Brown Extra Large blocks were not supplemented with additional medium based upon our assessment of the suitability of the amount already added. Both the green and brown medium sized blocks were supplemented with an additional 2 ml of medium, and both small block types received an additional 1 ml of medium. As a result, the total amount of medium added to each block type-size combination was: GXL = 94 ml, BXL = 70 ml, GL = 76 ml, BL = 49 ml, GM = 29 ml, BM = 19 ml, GS = 13 ml, BS = 10 ml.

All plants were cultured for 15 days at 20-22 ° C, under irradiation levels of about 1000 ft-c with a 16:8 light-dark cycle.

RESULTS AND DISCUSSION

Foam Block Dimensions

The small blocks averaged 3.24 and 3.16 cm in height for the green and brown foam types respectively. The mean longest top dimension for the green foam blocks was 2.12 cm, vs 2.16 cm for the brown. The mean shortest top dimension for the green foam blocks was 1.96 cm, vs 1.87 cm for the brown. This resulted in a mean surface area for the small green blocks of 34.7 cm², compared to 33.6 cm² for their brown counterparts. Comparable values for volume were 13.5 cm³ for the green and 12.8 cm³ for the brown. The resulting mean surface area to volume ratios for the green and brown small blocks were 2.58 and 2.63 respectively.

The medium sized blocks averaged 3.27 and 3.19 cm in height for the green and brown foam types respectively. The mean longest top dimension for the green foam blocks of this size class was 4.31 cm, vs 4.24 cm for the brown. The mean shortest top dimension for the medium sized green foam blocks was 2.38 cm, vs 2.44 cm for the brown. This resulted in a mean surface area for the medium green blocks of 64.2

cm², compared to 63.3 cm² for their brown counterparts. Comparable values for volume were 33.5 cm³ for the green and 33.0 cm³ for the brown. The resulting mean surface area to volume ratios for the green and brown medium sized blocks were 1.92 and 1.92.

The large sized blocks averaged 3.16 and 3.29 cm in height for the green and brown foam types respectively. The mean longest top dimension for the green foam types of this size class was 5.54 cm, vs 5.51 cm for the brown. The mean shortest top dimension for the large sized green foam blocks was 4.71 cm, vs 5.00 cm for the brown. This resulted in a mean surface area for the large green foam blocks of 117.1 cm², compared to 124.1 cm² for their brown counterparts. Comparable values for volume were 82.7 cm³ for the green and 90.5 cm³ for the brown. The resulting mean surface area to volume ratios for the green and brown large sized blocks were 1.42 and 1.37.

The extra large sized blocks averaged 3.40 and 3.45 cm in height for the green and brown foam types respectively. The mean longest top dimension for both foam types of this size class was 5.78 cm. The mean shortest top dimension for both of the extra large foam blocks types was also 5.78 cm. This resulted in a mean surface area for the extra large green foam blocks of 145.5 cm², compared to 146.7 cm² for their brown counterparts. Comparable values for volume were 113.7 cm³ for the green and 115.4 cm³ for the brown. The resulting mean surface area to volume ratios for the extra large green and brown blocks were 1.28 and 1.27 respectively.

Upon addition of media there was seepage from the block bottoms, especially from the brown foam. For the small and medium sized blocks, several rounds of pipetting up the excess and reapplying it to the block tops circumvented the problem. For the large and extra large size classes, however, this step was not required since the blocks maintained sufficient contact with the majenta bottoms to enable the reacquisition of any seepage.

The silicone treatments were relatively successful in localizing the foam blocks. On four occasions, after media addition, and often associated with manipulations involved in the placement of the experimental plantlets within the block slits, medium sized green blocks disattached from the vessel bottom. In these cases, the foam block material effectively gave way, leaving the silicone and a thin layer of foam still attached to the container bottom.

If the silicone attachment method is to be pursued, the following procedural modification may have some advantages. First, a thin layer of silicone should be applied to the bottom, and possibly the lowermost 1 cm along the four sides of the foam blocks. After this application has dried, additional silicone can be added to the center of the block bottom, and the adhesion between block and vessel made. There are two advantages to this approach. First, the application of a broader silicone layer to the block base results in an attachment substratum less likely to break or crumble. Second, this approach effectively water seals the bottom of the blocks, which should reduce the tendency for the supplied media to drain out through the block bottom.

Figure 1. Foam Block Surface Area and Volume Relationships

GSA = Green Foam Block Surface Areas

BSA = Brown Foam Block Surface Areas

GV = Green Foam Block Volumes

BV = Brown Foam Block Volumes

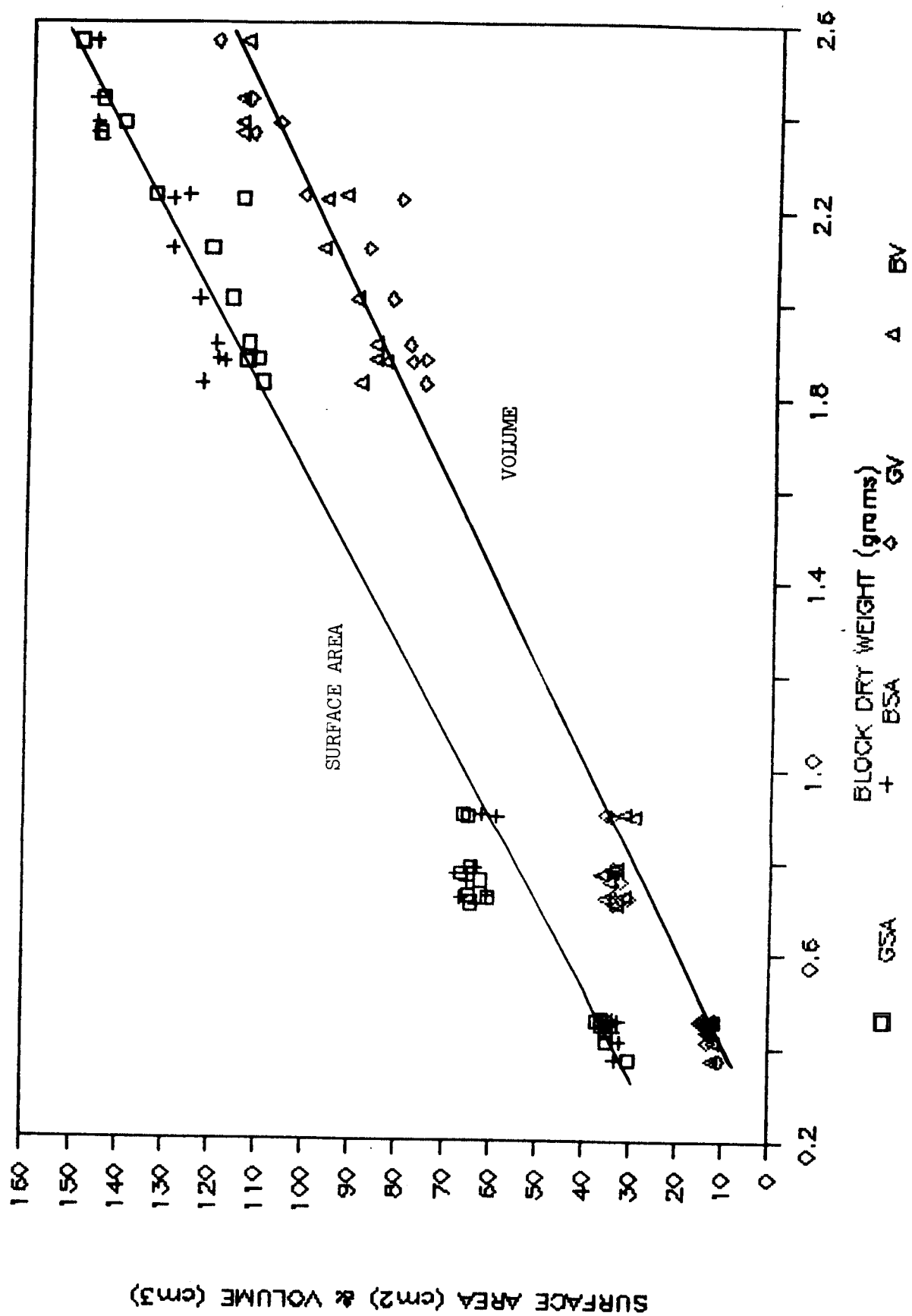


Figure 2a. Foam Block Surface Area to Volume Relationships:

Plotted vs Block Dry Weight

GSAV = Green Foam Block Surface Area:Volume

BSAV = Brown Foam Block Surface Area:Volume

GDWSAV = Green Foam Block Dry Weight X Surface Area:Volume

BDWSAV = Brown Foam Block Dry Weight X Surface Area:Volume

Figure 2b. Foam Block Surface Area to Volume Relationships:

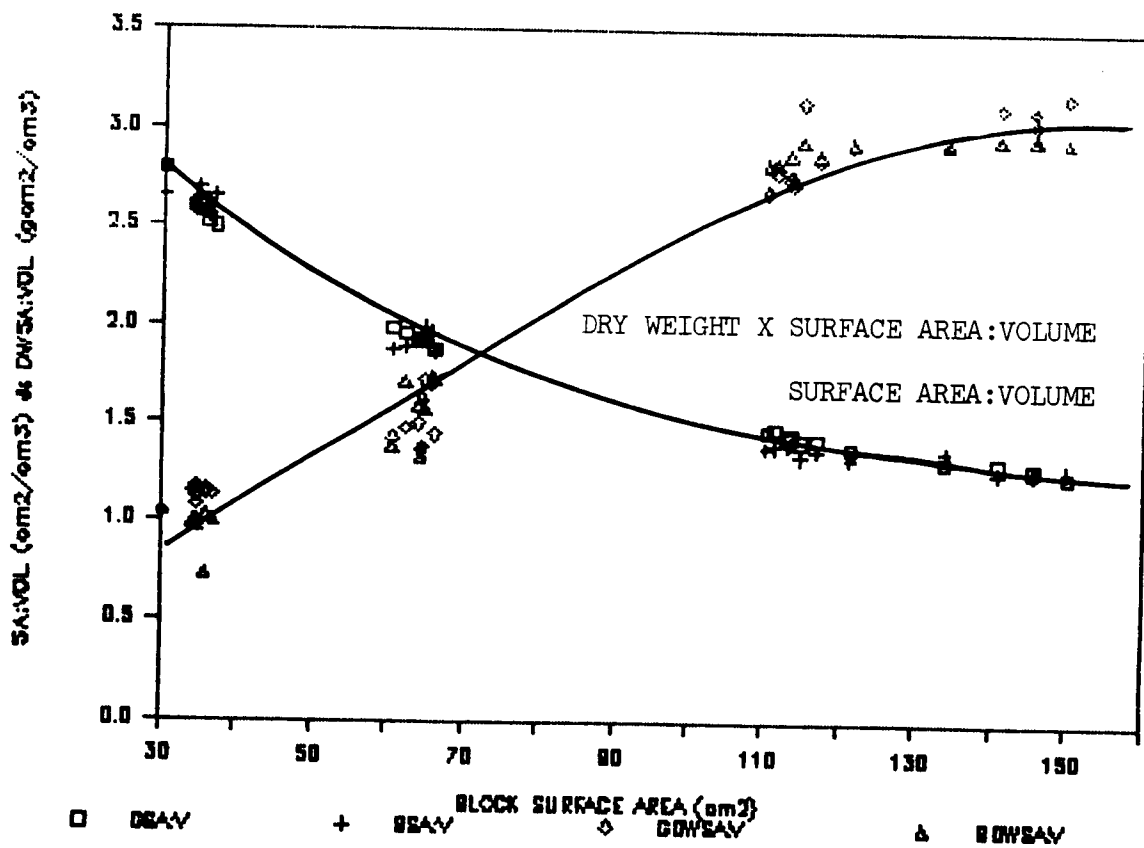
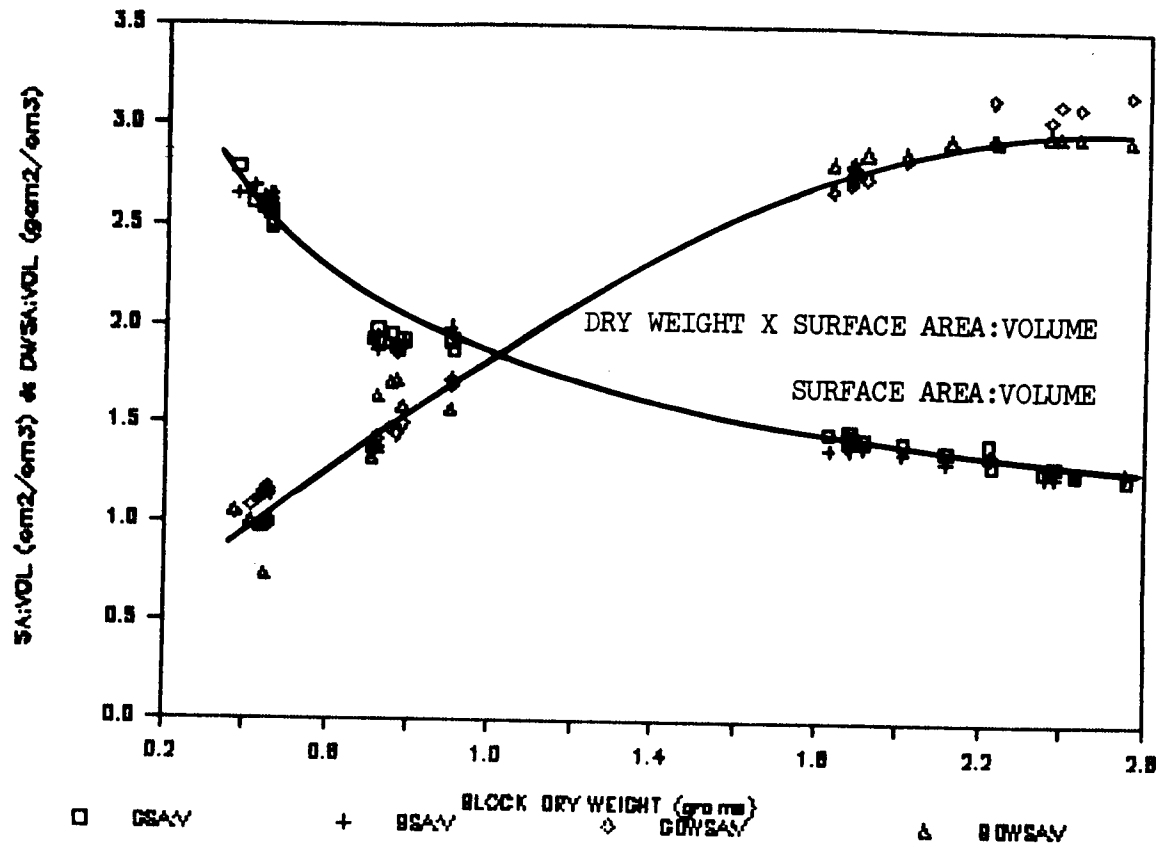
Plotted vs Block Surface Area

GSAV = Green Foam Block Surface Area:Volume

BSAV = Brown Foam Block Surface Area:Volume

GDWSAV = Green Foam Block Dry Weight X Surface Area:Volume

BDWSAV = Brown Foam Block Dry Weight X Surface Area:Volume



Both the small and medium sized foam blocks had but one of their six sides (the bottom) immediately adjacent to the majenta culture vessel. On the other extreme, the extra large blocks were both in contact with the majenta bottoms plus situated close to each of the four majenta side walls. In this case, only the top surfaces were completely open for evaporative purposes. The large sized blocks had both their top surface and two sides open for fullfledged evaporation. Clearly, the amount of surface area free for evaporative purposes would be expected to influence the liquid retention capacity for any given block shape. Foam blocks within the PGCs will be open only at their surfaces. The extra large block sizes are therefore the closest approximation to the eventual study conditions.

Figure 1 presents the data on the surface area and volume of the foam blocks as a function of size, represented here by dry weight. For the most part, both surface area and volume increase linearly with increasing size. All block shapes were rectangular, as will be those employed in the PGCs.

Figures 2a and 2b both present block surface area to volume relationships, but employ two different indicators of block size, dry weight (Figure 2a) and surface area (Figure 2b). In both cases, it can be seen that the surface area to volume ratio decreased with increasing block size. This is a critical point in terms of the media holding capacity of the blocks. The internal block environment would be expected to differ from the peripheral regions where water loss occurs via evaporation. If this is the case, the block interiors should be thought of as resevoirs which are gradually giving up their water to the exterior environment. As such, there is likely a gradient in moisture with a "high" in the block center and decreasing levels going outward. In reality, this effect is largely overwhelmed by the downward percolation of moisture within the foam blocks (see Foam Block Liquid Distribution Pattern Section).

Also presented in these figures are the surface area to volume values multiplied by block dry weights. At this point we merely include this as an additional means of modeling the surface area to volume relationship, but one which incorporates a block size factor.

Green and Brown Foam Characteristics

Figures 3a and 3b address the question of how much moisture is being lost from the blocks over the course of the study. Given a known initial addition of medium, and both the wet and dry weights of the majentas and blocks at the study termination, the amount of water lost during the study from each culture vessel (majenta) can be calculated (Figure 3a). Overall, it can be seen that the larger blocks, which received more medium initially, lost more moisture during the course of the study than the smaller blocks. When these data are viewed in terms of the amount of water lost per plant per day (Figure 3b), there appear to be larger per plant losses in the samller block cases, with a gradual leveling off as block size increases. We do not suggest that the experimental plantlets within the smaller blocks actually used more water. Rather, this relationship is

Figure 3a. Water Loss per Majenta vs Block Size

Figure 3b. Water Loss per Plant per Day

Figure 4a. Percent Primary and Secondary Root Formation:

All Data Lumped

GB + BB = Data from both Green plus Brown Foam Blocks

GB = Data from Green Foam Blocks

BB = Data from Brown Foam Blocks

Figure 4b. Length Attained by Primary and Secondary Roots:

All Data Lumped

GB + BB = Data from both Green plus Brown Foam Blocks

GB = Data from Green Foam Blocks

BB = Data from Brown Foam Blocks

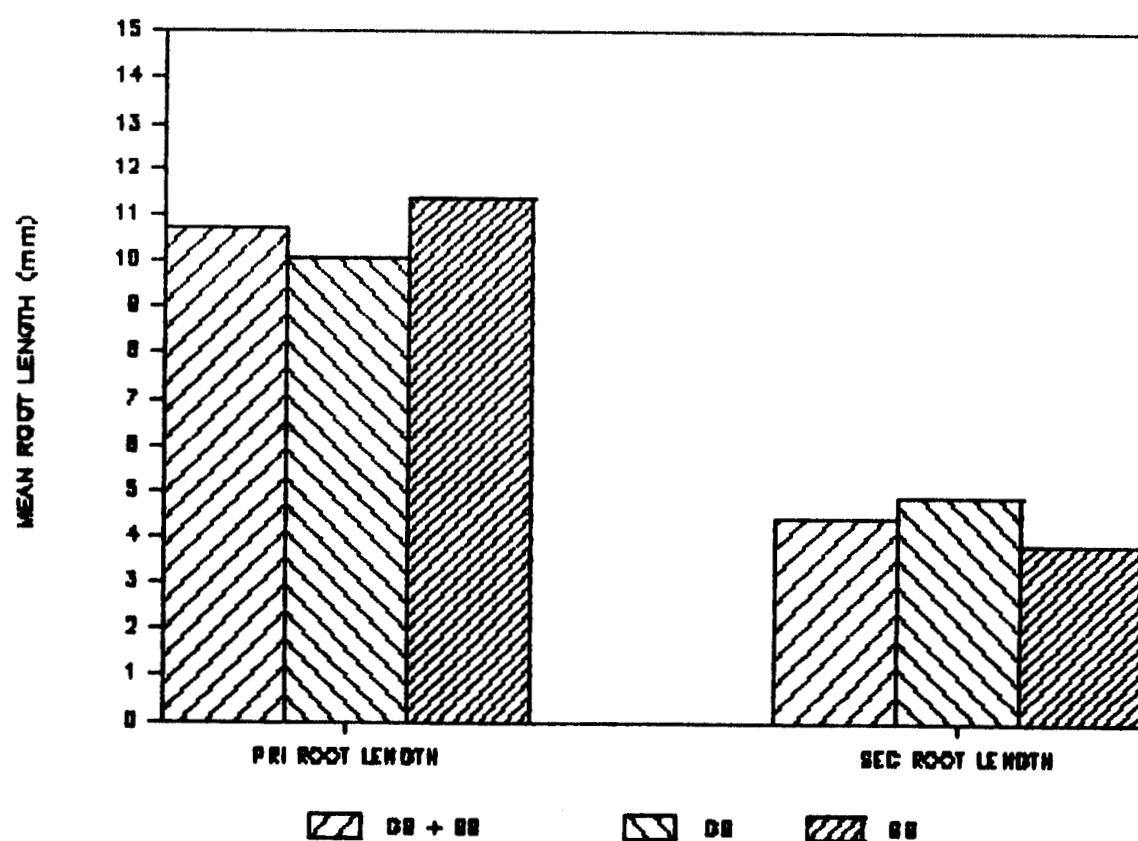
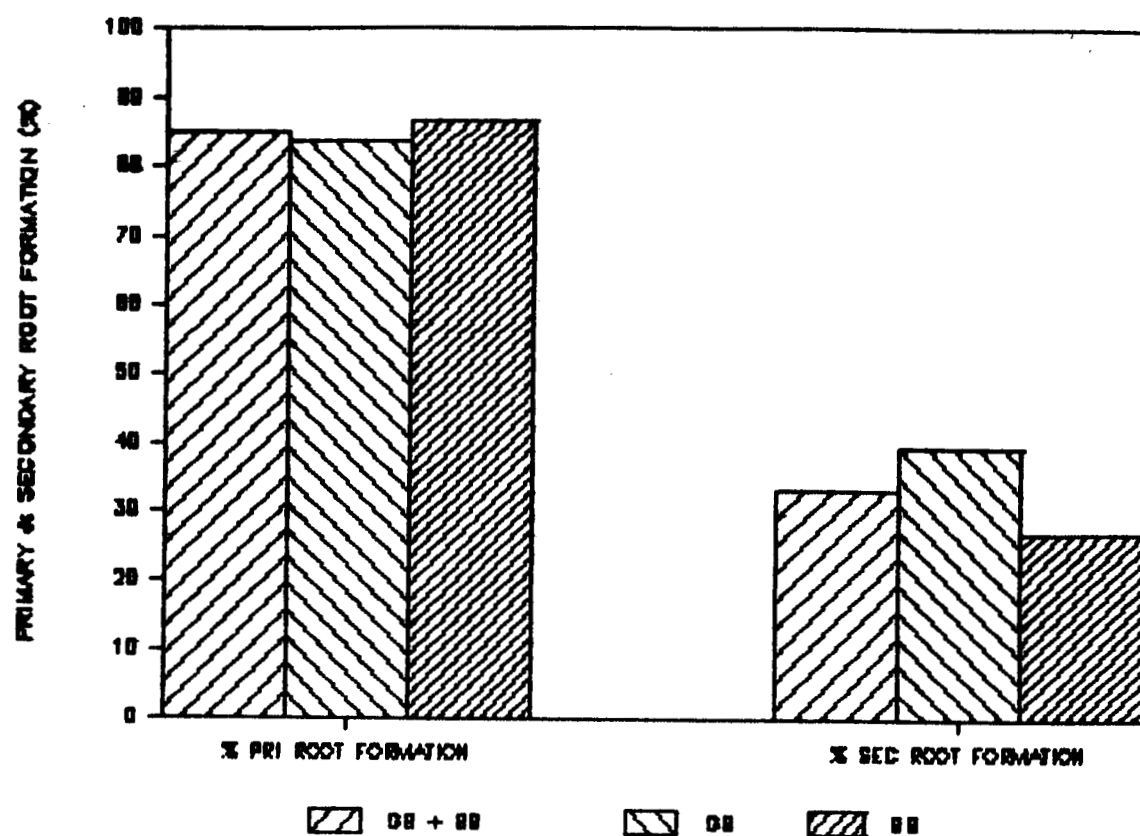


Figure 5a. Position Effects: Percent Primary Root Formation

FRONT = Plants Grown in the Front Slit Position

BACK = Plants Grown in the Back Slit Position

MIDDLE = Plants Grown in the Middle Slit Position

Figure 5b. Position Effects: Length Attained by Primary Roots

FRONT = Plants Grown in the Front Slit Position

BACK = Plants Grown in the Back Slit Position

MIDDLE = Plants Grown in the Middle Slit Position

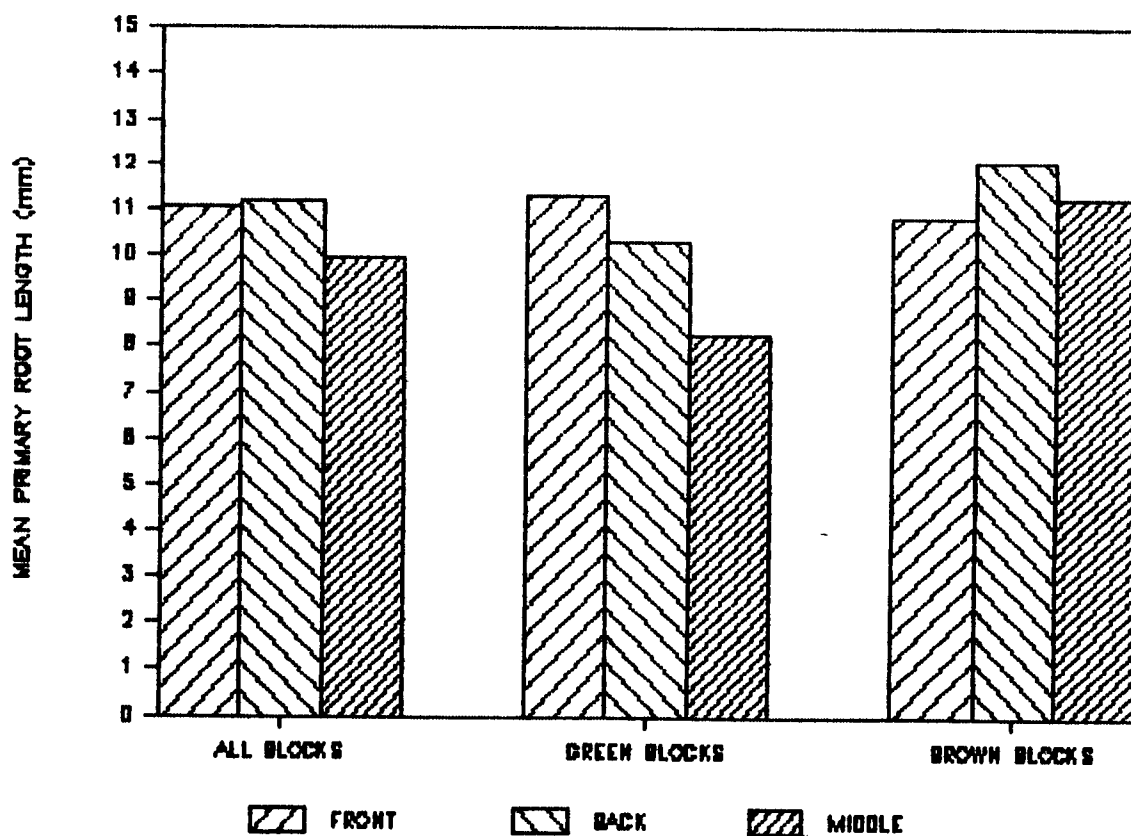
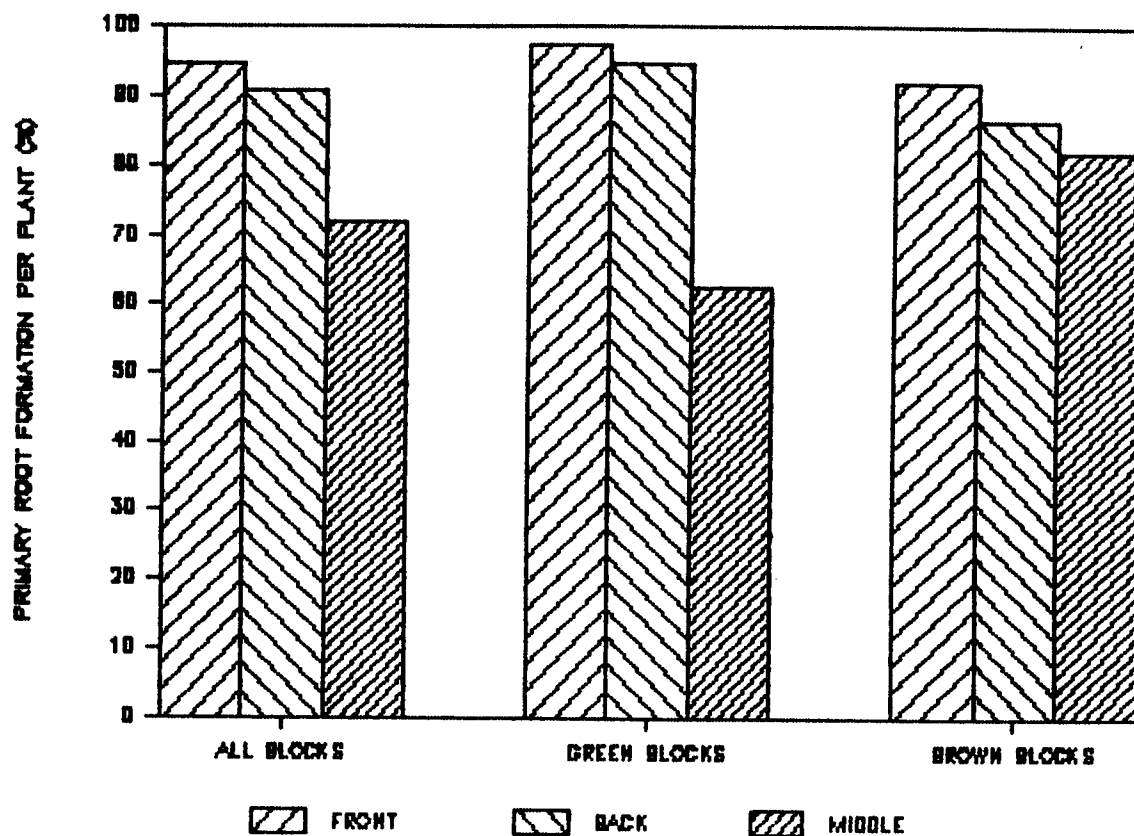


Figure 6a. Position Effects: Percent Secondary Root Formation

FRONT = Plants Grown in the Front Slit Position

BACK = Plants Grown in the Back Slit Position

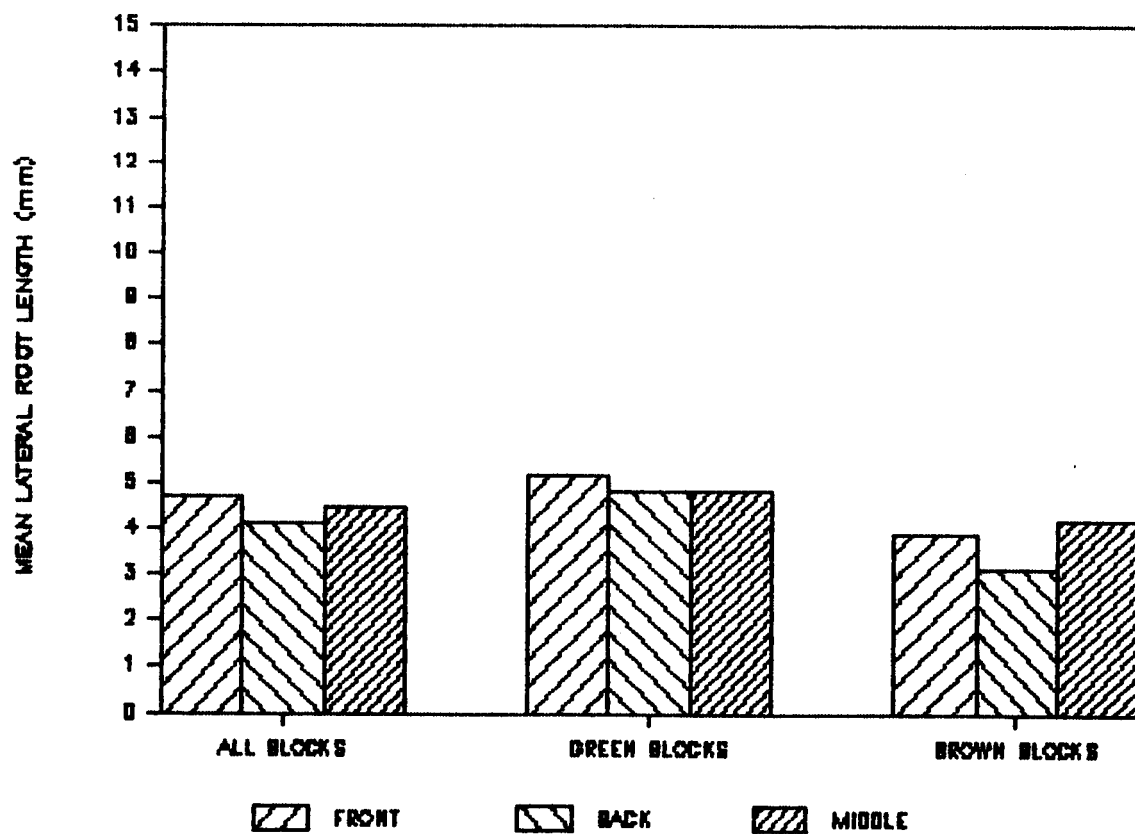
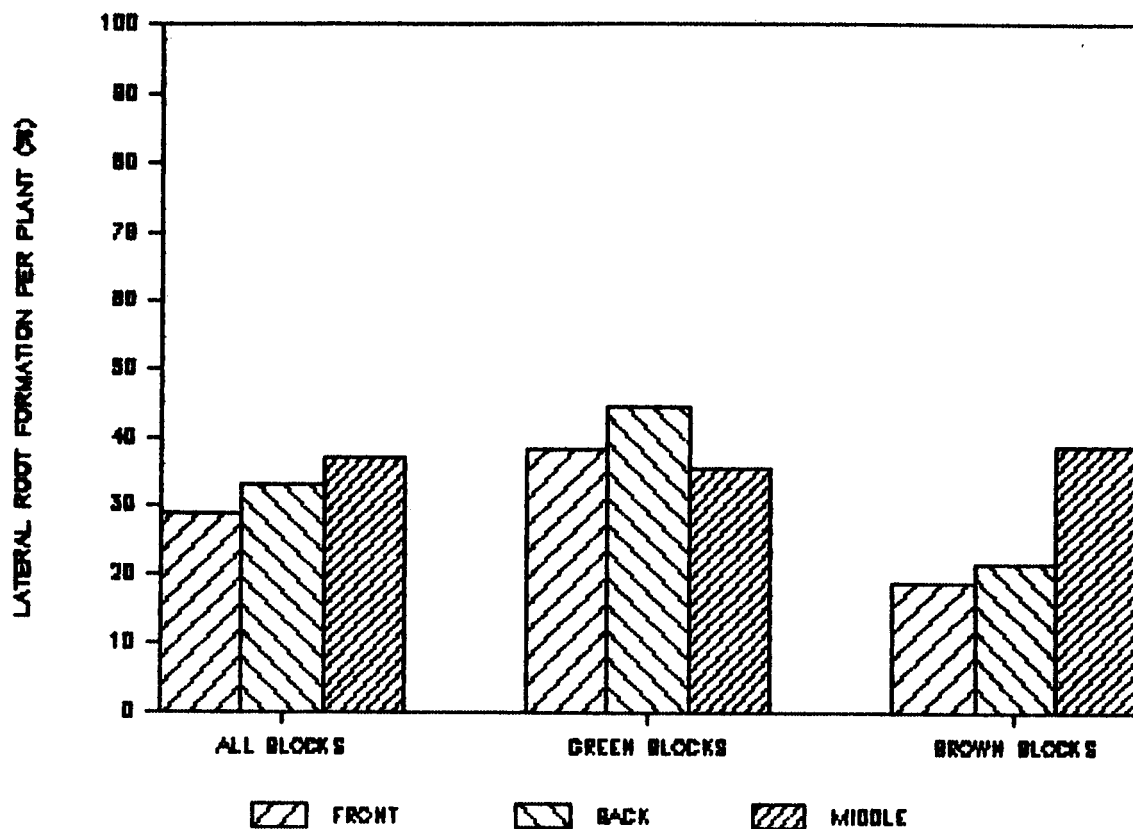
MIDDLE = Plants Grown in the Middle Slit Position

Figure 6b. Position Effects: Length Attained by Secondary Roots

FRONT = Plants Grown in the Front Slit Position

BACK = Plants Grown in the Back Slit Position

MIDDLE = Plants Grown in the Middle Slit Position



interpreted as reflecting the relative independence of block water loss relative to the number of plantlets employed. Block water loss appears to be primarily a function of initial medium addition, the more added the more lost. Clearly, the majenta containers are not air tight.

Having said that, there is an indication of a greater water loss in the small and medium brown blocks vs their green counterparts (Figures 3a and 3b). Presumably, this reflects the greater porosity of the brown foam type. This difference is not evident for the large and extra large size blocks possibly reflecting a threshold effect with respect to the amount of medium added and then lost.

Primary and Secondary Root Formation

Figures 4-7 present histograms representing mean values for "all" the blocks (= green + brown) as well as for the green and brown block subsets. Both green and brown blocks formed primary roots 84% and 86% of the time respectively (Figure 4a). In terms of primary root length, those formed in the brown blocks averaged 11.4 mm vs 10.0 mm for those from the green blocks.

With respect to secondary root formation, however, percent formation was greater in the green blocks (39% vs 27%; Figure 4a), and the mean length of those formed was 4.9 mm vs 3.8 mm (Figure 4b).

Conceivably, high water or high nutrients result in decreased frequency of primary root formation, at least for the green blocks which contained appreciably more medium than the brown blocks. Consider that the position effects for the large and extra large blocks should be less if light is the primary cause since for each middle row, two of the three plantlets are actually in a side position. This may indicate that the middle position effect is more a function of the water/gas/nutrient regime within the blocks than irradiance.

Figures 5 and 6 break up the data into three position subclasses, front, middle and back. Both front and back positions represent nitex-lined slits about 1 cm in from the blocks' edges, and as such can be thought of as peripheral positions. The middle slits were placed within the center of each block. Primary root formation was lowest in the middle positions for both green and brown block types (Figure 5a). The effect was more pronounced for the green blocks. In addition, primary roots were very much shorter at the middle position for the green blocks (Figure 5b), although intermediate for the brown blocks. Our current thinking is that the higher moisture level within the block interiors (middle positions) affected the length and frequency of root formation.

Figures 6a and 6b present comparable data for lateral root formation. Other than the already mentioned increase in secondary root development in the green vs brown blocks, little can be extracted from these graphs. However, note that the lowest frequency of lateral root formation in the study (and the shortest laterals as well) were found in the peripheral (front and back) regions of the brown blocks

Figure 7a. Nitex Mesh Size Comparisons:

Percent Primary Root Formation

GB + BB = Data from both Green plus Brown Foam Blocks

GB = Data from Green Foam Blocks

BB = Data from Brown Foam Blocks

Figure 7b. Nitex Mesh Size Comparisons:

Length Attained by Primary Roots

GB + BB = Data from both Green plus Brown Foam Blocks

GB = Data from Green Foam Blocks

BB = Data from Brown Foam Blocks

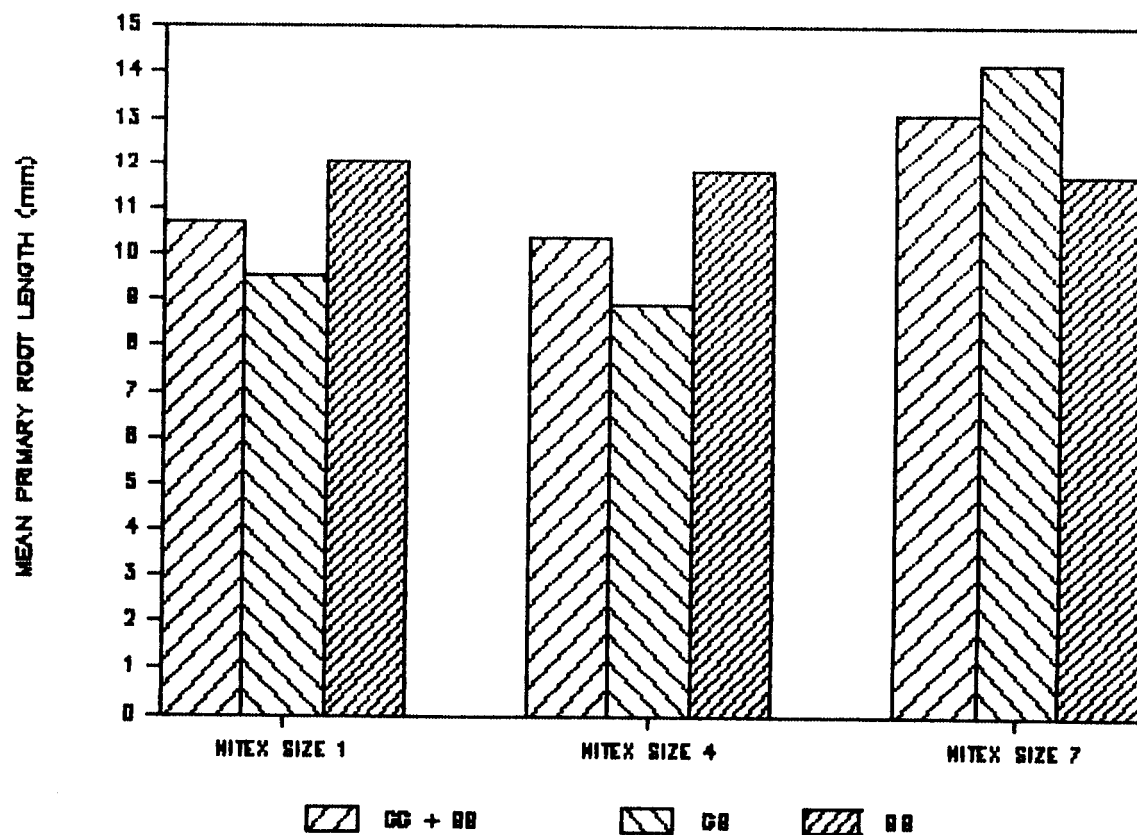
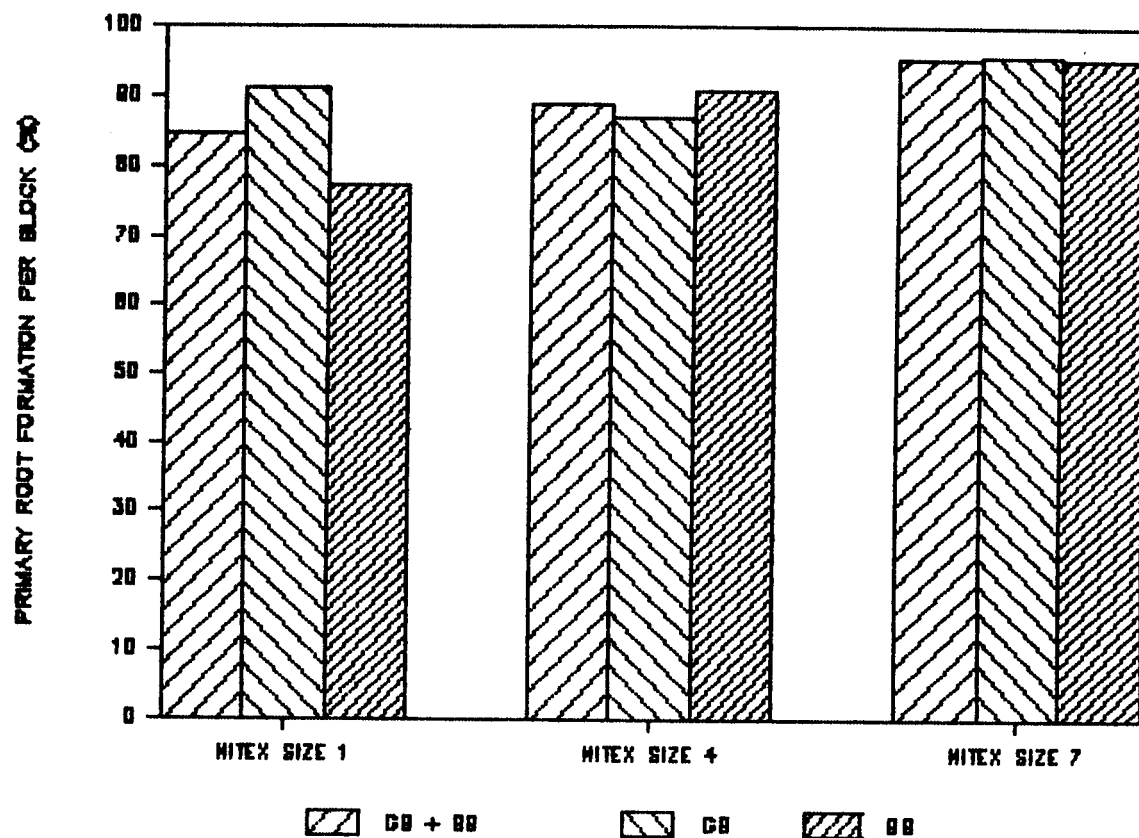


Figure 8a. Nitex Mesh Size Comparisons:

Percent Secondary Root Formation

GB + BB = Data from both Green plus Brown Foam Blocks

GB = Data from Green Foam Blocks

BB = Data from Brown Foam Blocks

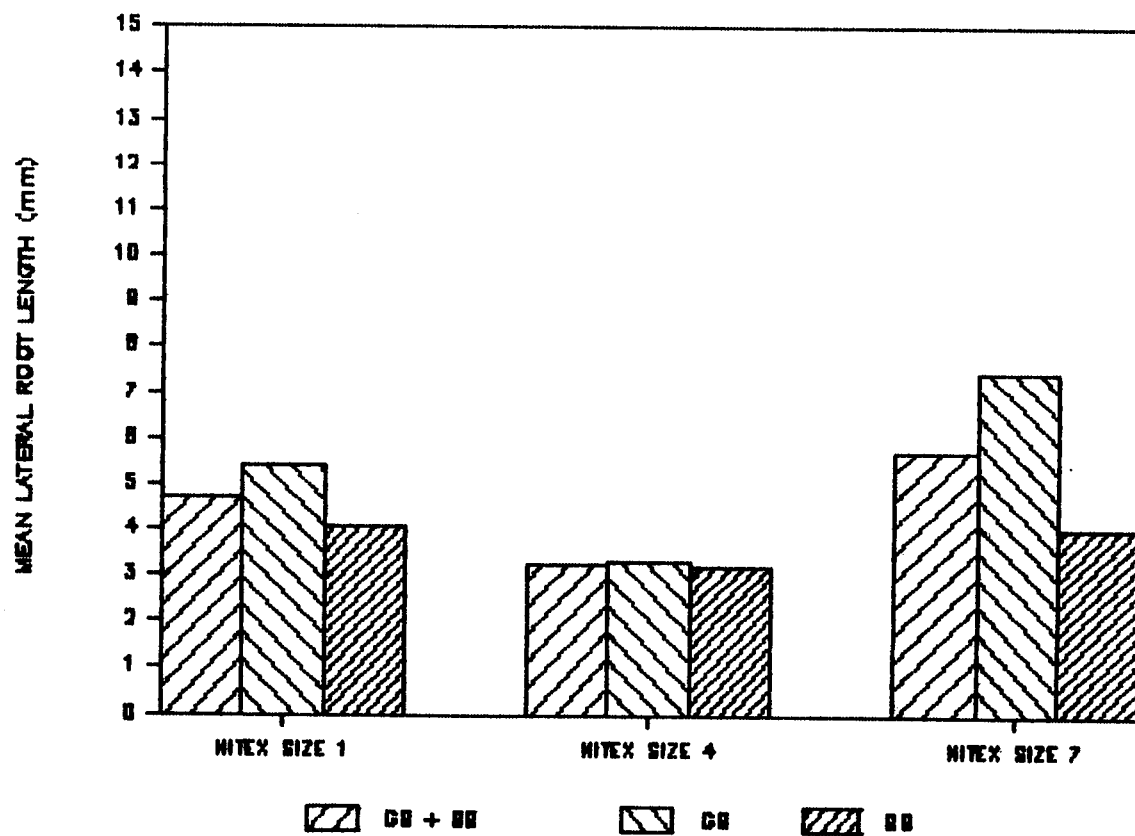
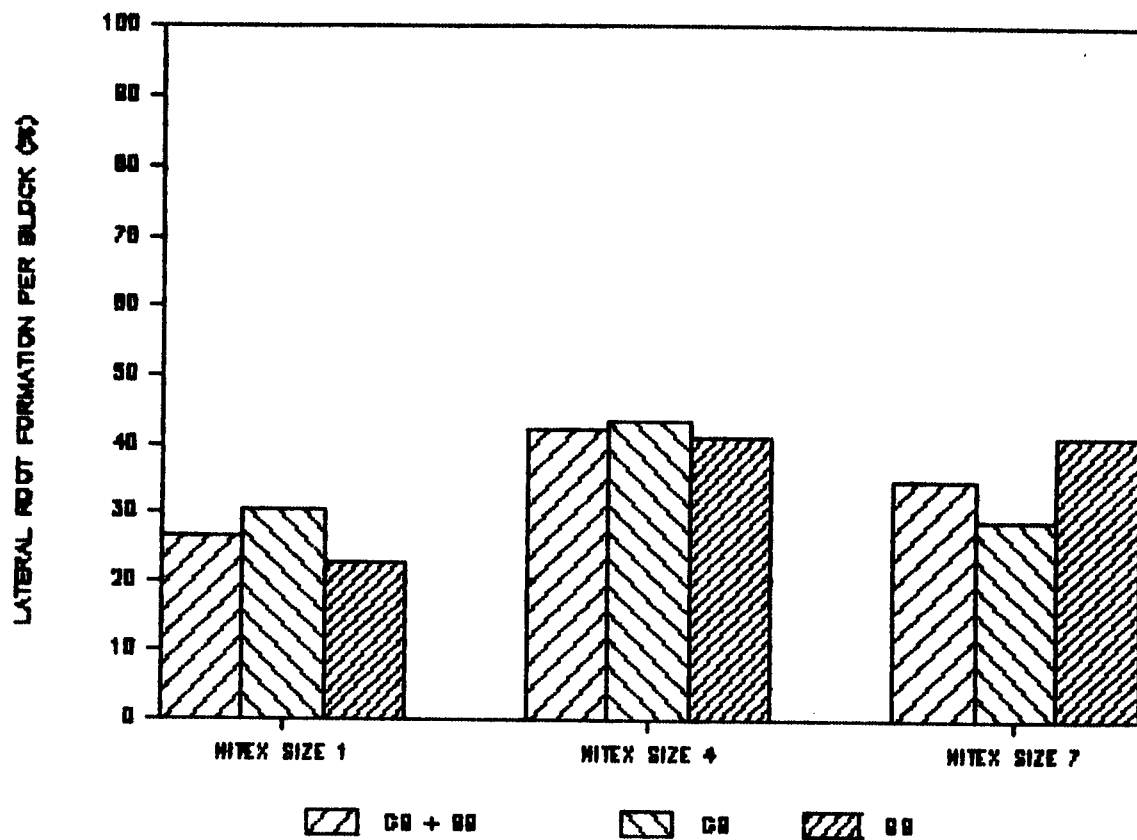
Figure 8b. Nitex Mesh Size Comparisons:

Length Attained by Secondary Roots

GB + BB = Data from both Green plus Brown Foam Blocks

GB = Data from Green Foam Blocks

BB = Data from Brown Foam Blocks



(Figure 6a). Presumably, the combination of the lower moisture content of these blocks plus the drier block regions resulted in an environment less conducive to secondary root propagation.

Figures 7 and 8 present mean values for three of the nitex mesh values (1, 4, 7). Nitex size 7 resulted in 96% and 95% of the plants forming primary roots for green and brown blocks respectively (Figure 7a). The effect of mesh size was most pronounced within the brown blocks. Conceivably, the larger mesh size resulted in lower medium/water availability. In terms of primary root length, Nitex 7 clearly gave the largest primary roots obtained (about 14.3 mm) for the green blocks (Figure 7b). All Nitex sizes gave equivalent primary root lengths in the brown foam blocks.

The picture is a bit fuzzier relative to secondary root formation. For brown blocks, both nitex sizes 4 and 7 gave equivalent percent formations for laterals (41%; Figure 8a), and the entire range of lateral root lengths are not thought to be significantly different (Figure 8b).

For the green blocks, the greatest number of laterals were found with Nitex 4 (Figure 8a), but they were the shortest (Figure 8b). The longest secondary roots found, however, were with green blocks employing Nitex size 7. This may reflect an aeration factor.

CONCLUSION

Both floral foam types were found to be suitable for daylily root growth studies employing tissue culture-derived plantlets. The media holding capacities of the green and brown foams were found to be more than sufficient, and in fact provide the option of easily employing varying moisture regimes. In terms of material characteristics, no restrictions were encountered.

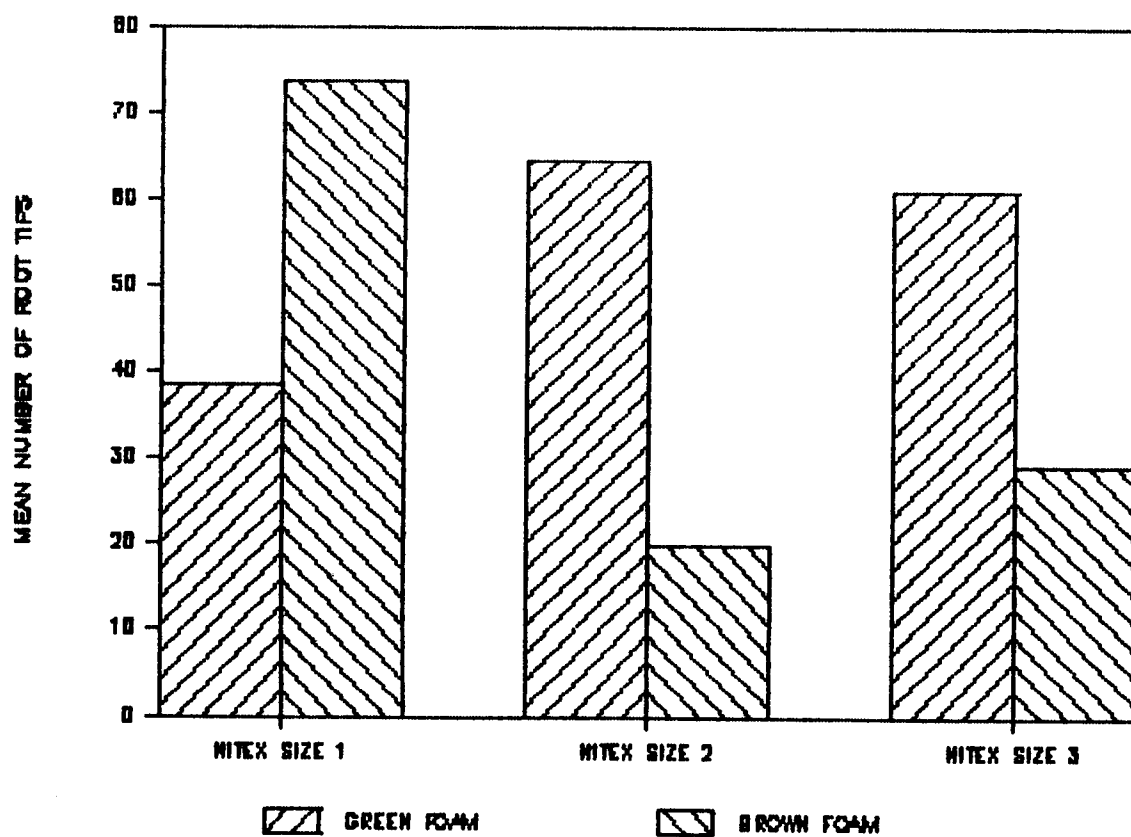
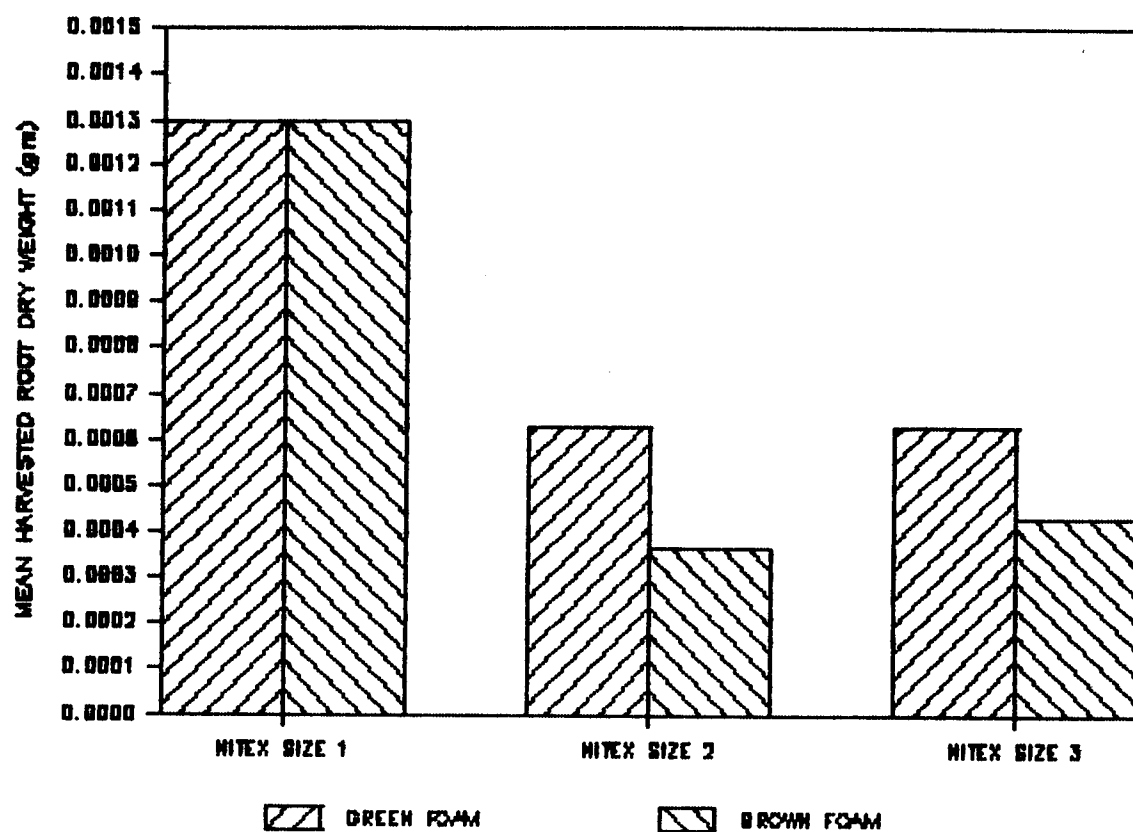
The extra large size blocks proved most suitable for experimental purposes. They eliminated the need to attach the blocks to the container with silicone. Also removed is the problem of medium seepage from the block bottom. Addition of media merely required pouring premeasured amounts onto the blocks and permitting the liquid to equilibrate throughout the block. The proximity of the extra large blocks to the majenta walls also most closely approximates the conditions within the PGC units.

We believe the two foam types capable of receiving varying amounts of media. Given equal additions, the primary difference between the green and brown foams lies in their porosities, a factor which may be useful in experiments investigating the effects of aeration on root formation.

All seven nitex mesh sizes tested gave adequate results. There was no evidence of root hair entanglement in the foam matrix. Neither were there indications of toxic effects attributable to the nitex (nor the foam blocks). Mesh size 7 (the largest tested) appeared to give the best results. The reasons are thought to be due to air-water interactions within the foam block interiors.

Figure 9a. Haplopappus gracilis Foam-Nitex Comparison Study:
Mean Dry Weights of Harvested Roots per Plant

Figure 9b. Haplopappus gracilis Foam-Nitex Comparison Study:
Mean Number of Root Tips per Plant



b. Haplopappus gracilis FOAM/NITEX EVALUATION STUDY

This investigation was a scaled down Haplopappus counterpart to the above daylily study. Three majentas containing medium-sized (as given above) green blocks plus three containing medium-sized brown blocks were established. Nitex sizes 1, 2 and 3 were tested. The slender habit of Haplopappus roots eliminated nitex sizes 4-7 from consideration. Each medium sized block had three nitex-lined planting slits containing one plant per slit. The experiment was conducted under continuous (ca 400 ft-c) irradiation at 22 C for 14 days.

After the experimental interval roots were severed from the experimental plants and counted. The mean dry weight values for these harvested roots are presented in Figure 9a. Both green and brown foam blocks containing nitex size 1 resulted in the greatest amount of root production on a dry weight basis (0.0013 gm). In contrast, both nitex sizes 2 and 3 produced lower, but virtually identical values in terms of total dry weight obtained per plant. In both cases, the green foam outperformed its brown counterpart.

When the mean number of root tips generated per plant are plotted (Figure 9b) the pattern seen above for nitex sizes 2 and 3 is repeated. Both mesh sizes gave similar results, and again, the green foam outperformed its brown counterpart, even moreso than with the dry weight criterion. With regard to nitex size 1, the brown foam again outperformed all nitex size 2 and 3 treatments. However, the mean number of root tips generated with nitex size 1 in the green foam was less than any other green foam treatment.

Upon separation of the root masses from the nitex liners, an occasional root was found to have penetrated nitex size 3, thereby eliminating it from consideration. If the data on nitex sizes 1 and 2 were substantially added to and the same pattern maintained, the selected mesh size would depend upon the primary criterion selected, either dry weight or the number of harvestable root tips. If root dry weight is deemed to be of upmost importance, nitex size 1 would be chosen. However, if the number of root tips generated is given priority, and if the green foam type is to be used, these very preliminary data suggest that nitex size 2 may yield higher numbers of root tips.

c. FOAM BLOCK LIQUID DISTRIBUTION PATTERNS

The question arises as to how liquid media becomes distributed within the foam blocks. To address this question, we have performed a series of block cutting studies. In all cases, whether the foam block being cut was of the small, medium, large, extra-large, or of the PGC size class, the blocks were divided into 27 subsections of approximately equal volume. Each block was divided into top, middle and bottom thirds. Within each of these thirds, three widthwise plus three lengthwise cuts were made, resulting in nine smaller subsections. These 27 subsections constitute the minimum number capable of illuminating both the vertical heterogeneities and any

Figure 10a. Foam Liquid Distribution Patterns:

Extra Large Sized Green Foam After 61 Hours Drying

Figure 10b. Foam Liquid Distribution Patterns:

Extra Large Sized Brown Foam After 61 Hours Drying

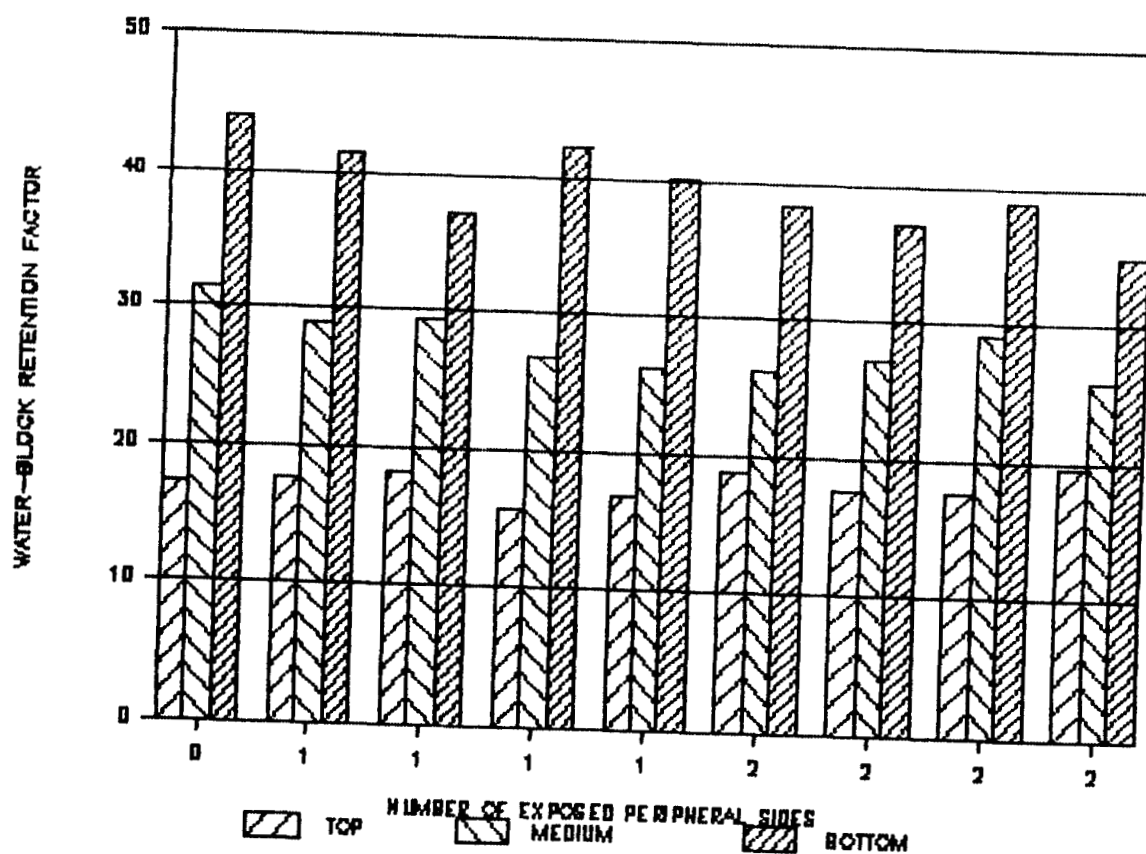
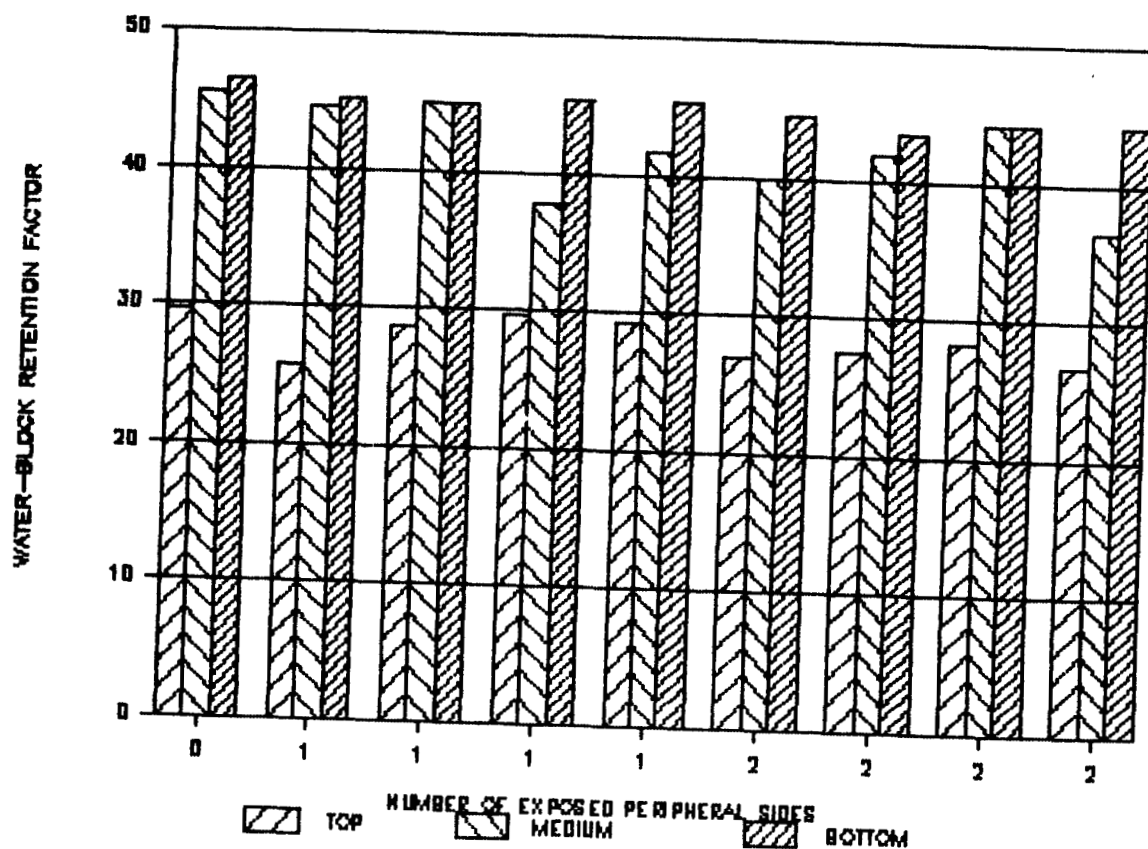
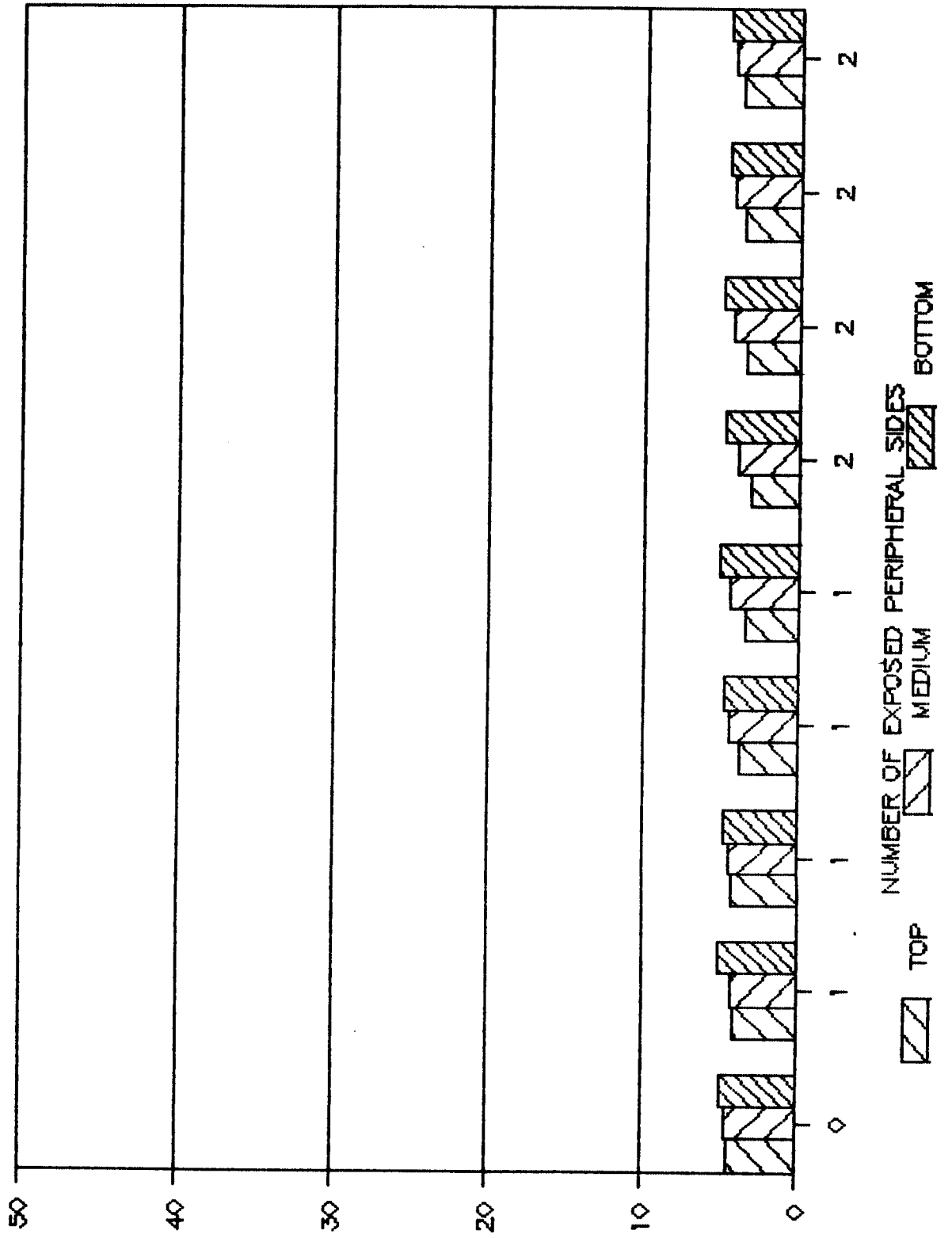


Figure 11. Foam Liquid Distribution Patterns:

Small Sized Green Foam After 49 Hours Drying

WATER-BLOCK RETENTION FACTOR



differences between areas (subsections) with exposed peripheral sides which presumably would lose moisture to a greater degree than the completely surrounded middle located subsections.

Matching pairs of green and brown foam blocks were supplied with equal amounts of either distilled water or liquid media. The blocks were then exposed to various drying intervals after which they were quickly divided up into their 27 subsections and wet weights obtained for each. They were then dried to a constant dry weight (usually 48 hours at 60° C). By dividing the initial subsection wet weights by the subsections' dry weights, a value termed the "retention factor" was obtained. The retention factor represents the level of moisture retained per subsection based upon the foam block's dry weight. This procedure therefore provides an index of retained moisture which corrects for different sized subsections.

Figures 10a and 10b present a typical data set collected from a matched pair of extra large sized green and brown foam blocks respectively. Both blocks were dried within majentas with their tops off for 61 hours. The primary pattern evident is the gradient of increasing moisture content going downward through the blocks. This effectively mimics the situation in soils, where water gradually percolates downward (a gravity related phenomenon). It is also evident that in all cases, the degree of moisture retention in green subsections always exceeded that of their brown counterpart subsections. This has been a consistent result throughout our studies, and reflects the lower degree of porosity and correspondingly higher water retention capacity of the green foam.

Figure 11 presents the results from a study involving a small sized green foam block subjected to a 49 hour drying interval. The Y-axis scale matches those of Figures 10a and 10b for comparison purposes. Note that the general pattern of the least moisture being found in topmost subsections, and increasing levels of moisture going downward is retained even as the block approached complete dryness. The brown counterpart of this block had completely lost all moisture by 49 hours.

During the interval of 6/22/88 - 6/27/88 we conducted a simulated shuttle flight experiment using the facilities of the Life Sciences group at the Kennedy Space Center in Florida. Green foam blocks were fitted to PGCs 2, 3, 4, 5 and 6. After the plant material was harvested at the end of the five day growth interval, the PGC foam blocks were cut up into the 27 subsections and processed. We wish to thank the personnel of Bionetics as well as the participants in the NASA sponsored SLSTP course for their assistance in this endeavor.

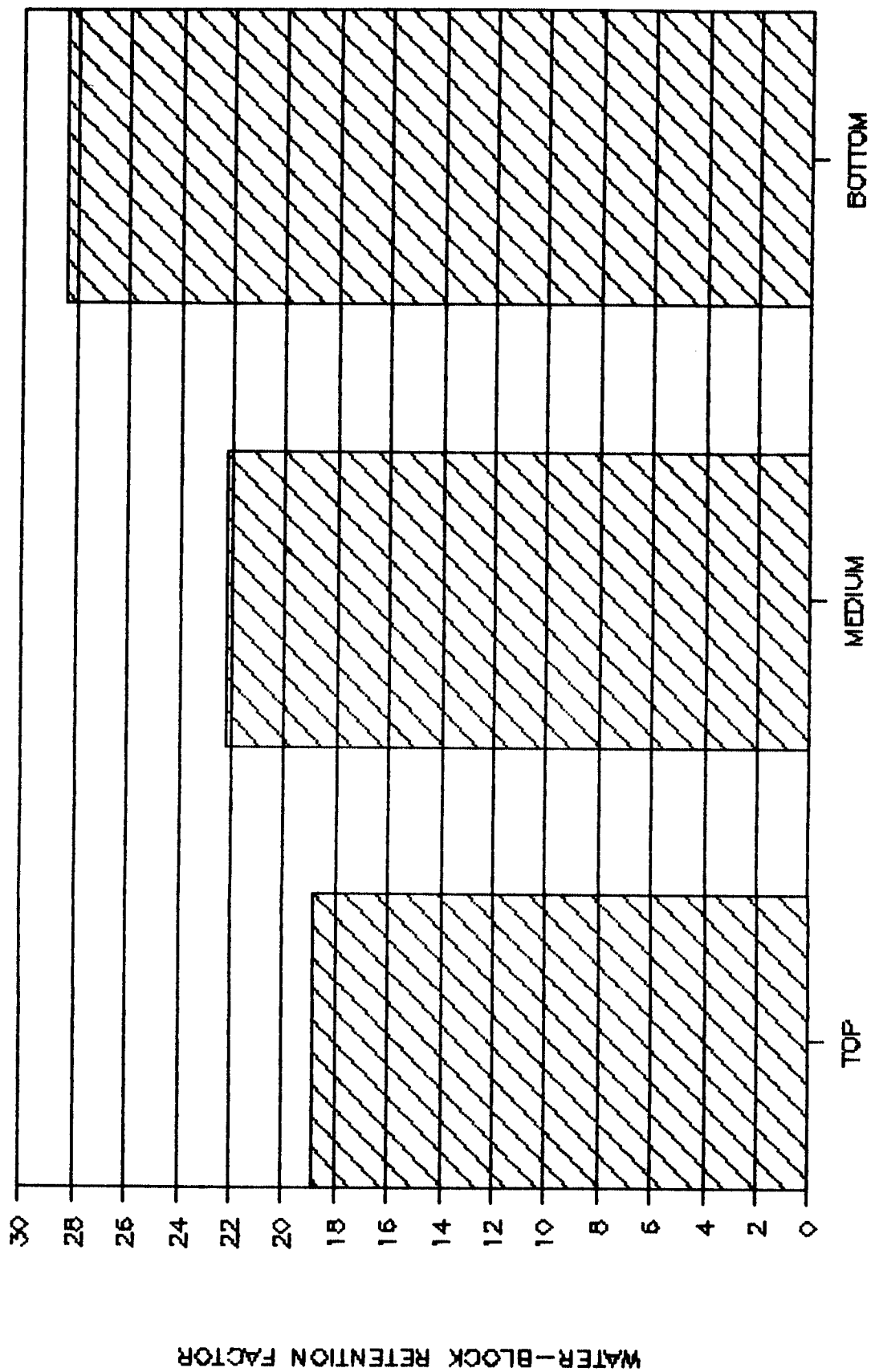
Figures 12, 13 and 14 summarize these findings by lumping and averaging the data from each of the 27 subsection positions for PGCs 2, 3, 4, 5 and 6. In Figure 12, only the top, middle and bottom subsection means from the centermost part of the foam blocks are presented. These subsections have no exposed peripheral sides, and clearly reflect the usual pattern of increasing moisture going downward in the foam blocks.

Figure 12. Foam Liquid Distribution Patterns:

KSC EDU Simulation Run 6/22/88 - 6/27/88

Overall Means for PGCs 2, 3, 4, 5, 6.

Middle Position Subsections - No Exposed Sides



MIDDLE POSITION: NO PERIPHERAL SIDES

Figure 13. Foam Liquid Distribution Patterns:

KSC EDU Simulation Run 6/22/88 - 6/27/88

Overall Means for PGCs 2, 3, 4, 5, 6.

Peripheral Position Subsections - One Exposed Side

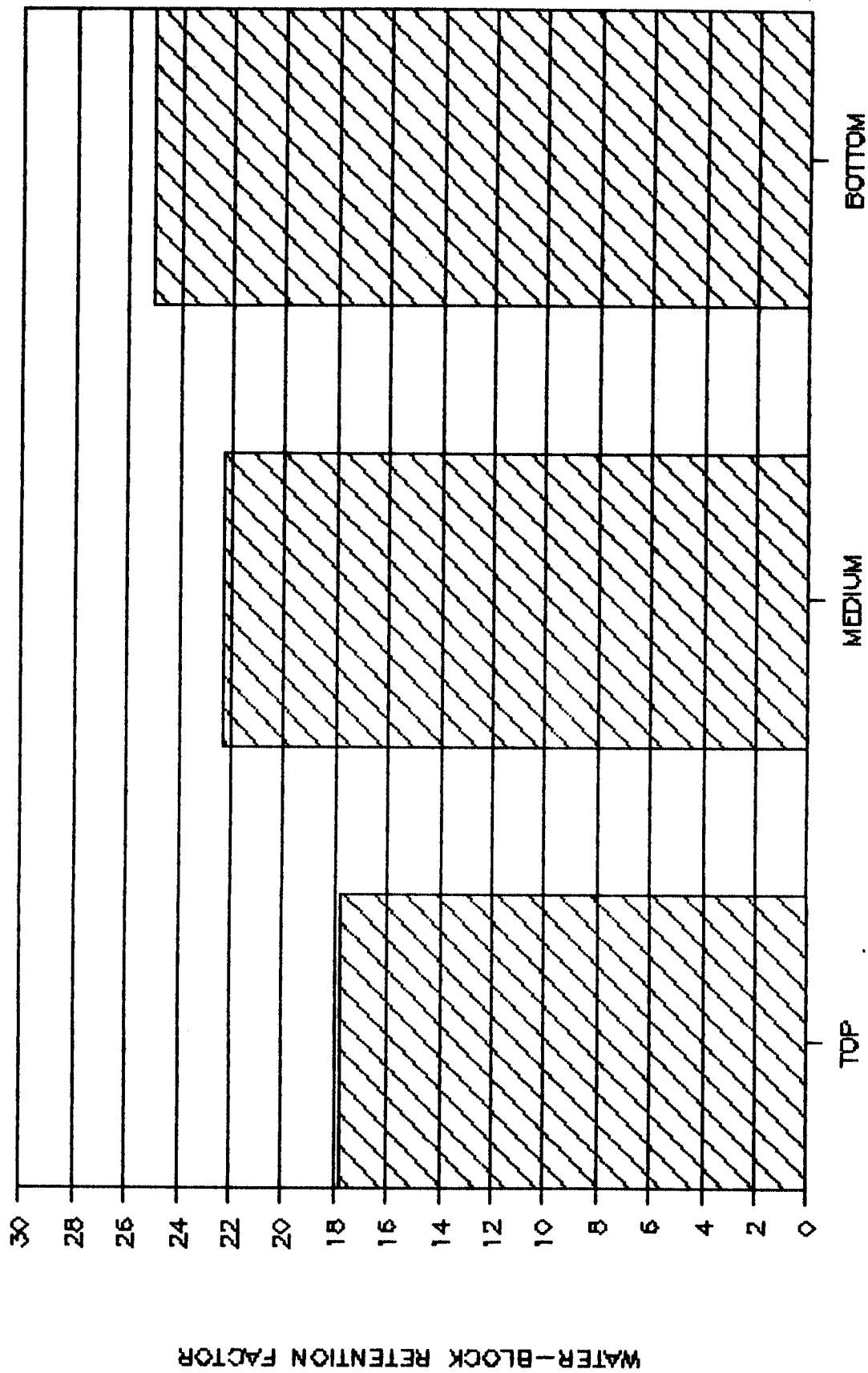


Figure 14. Foam Liquid Distribution Patterns:

KSC EDU Simulation Run 6/22/88 - 6/27/88

Overall Means for PGCs 2, 3, 4, 5, 6.

Peripheral Position Subsections - Two Exposed Sides

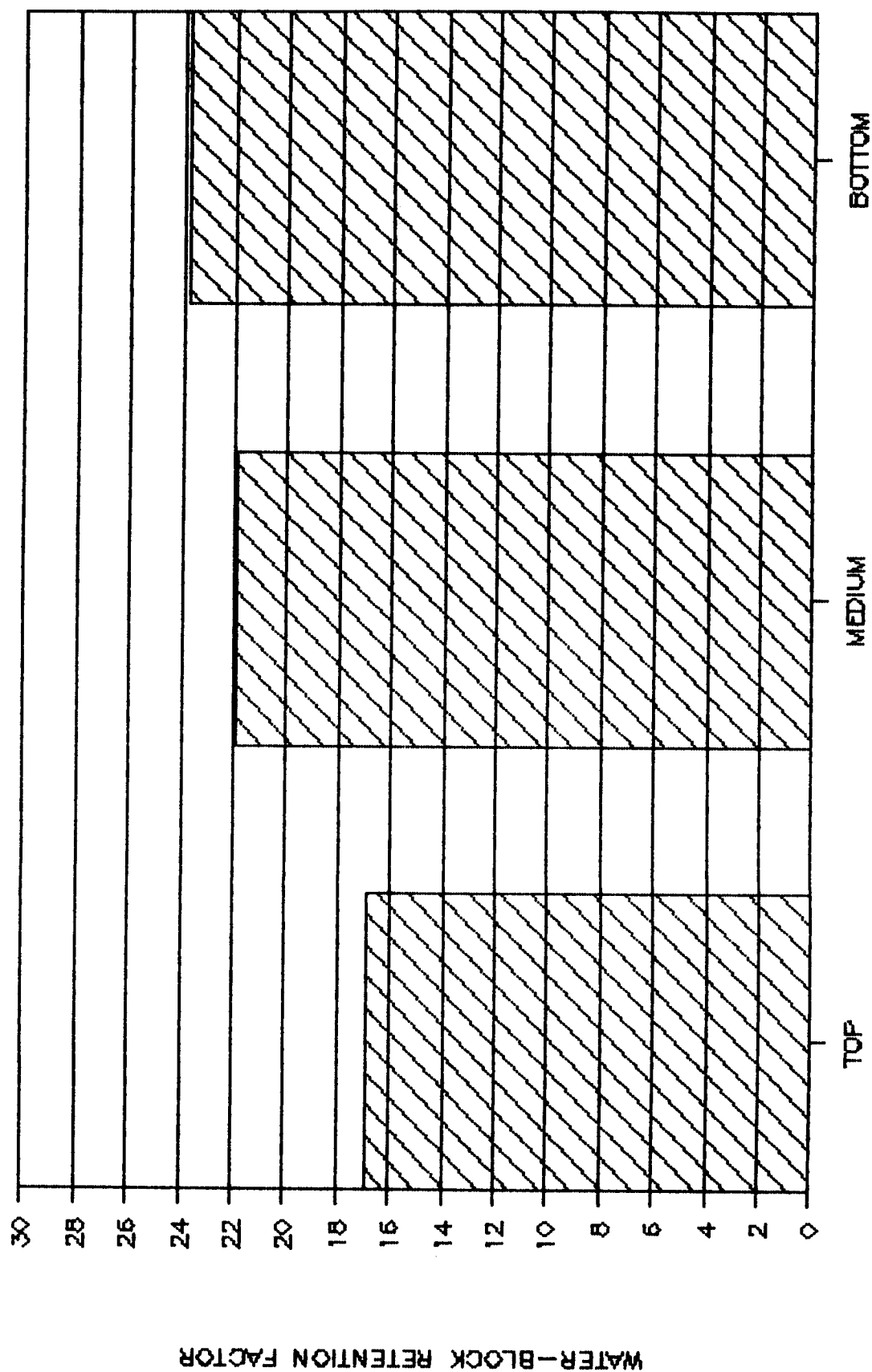


Figure 13 is similar except that the subset of subsections averaged consists only of those possessing one peripheral side exposed to the wall of the culture vessel (in this case the PGC base). And in Figure 14 the data subset consists of those subsections possessing two peripheral sides exposed to the PGC base. In both cases, the usual pattern described above is clearly evident.

The number of exposed peripheral sides is of much less significance than the downward percolation process in describing the liquid distribution patterns within the foam blocks. Nevertheless, a careful examination of Figures 10a, 10b, 12, 13 and 14 reveals a discernable tendency for moisture retention to decrease with increasing peripheral exposure. The only surprise is in the magnitude (barely perceptible) of the effect.

3. PGC-BASED STUDIES

We have tested various types of experimental plant material in the Stony Brook based EDU apparatus. Figures 15 and 16 presents the root tip production results from an eight day simulation run conducted during the interval of 5/11/88 - 5/19/88.

Each PGC foam block contains four nitex-lined planting slits. This permits the deployment of up to four different populations of experimental material within any one PGC. In this instance, two planting slits were devoted to daylily tissue culture derived plantlets, and two to Haplopappus gracilis seedlings.

One of the daylily subpopulations was grown under the influence of the antigibberellin, ancymidol, resulting in a broad (mature) leaf morphology. The second daylily subpopulation consisted of "normal" daylily plantlets possessing narrower (first year) blades. As can be seen in Figure 15, the ancymidol treated plantlets outperformed their "normal" counterparts in each of the five PGCs. This result has been repeated several times.

On occasion, we observed daylily root tips penetrating the nitex mesh size 7. In other studies (not reported here), there were also rare penetrations of nitex size 6 by daylily root tips. We therefore currently recommend the use of nitex mesh size 5 for daylily root formation investigations.

Of the two Haplopappus subpopulations, one consisted of young seedlings and the other of older (and larger) seedlings. Plants from both populations produced numerous root tips, with the older seedlings outperforming the younger seedlings by a factor of three.

Figure 15. Daylily PGC Root Tip Production:

Stony Brook EDU Run 5/11/88 - 5/19/88

2G = PGC Position 2 - Green Foam

3B = PGC Position 3 - Brown Foam

4G = PGC Position 4 - Green Foam

5B = PGC Position 5 - Brown Foam

6G = PGC Position 6 - Green Foam

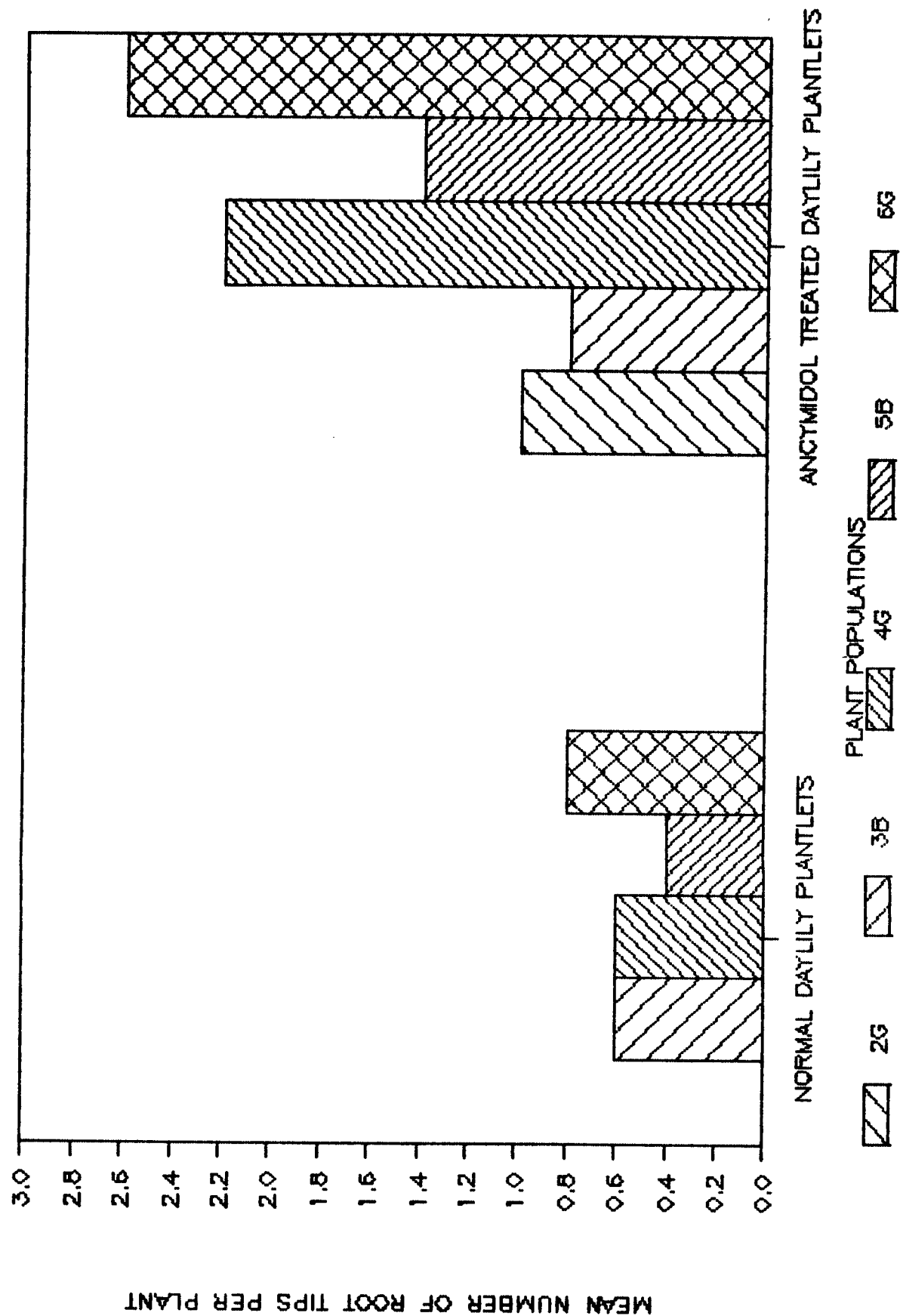


Figure 16. Haplopappus gracilis PGC Root Tip Production:

Stony Brook EDU Run 5/11/88 - 5/19/88

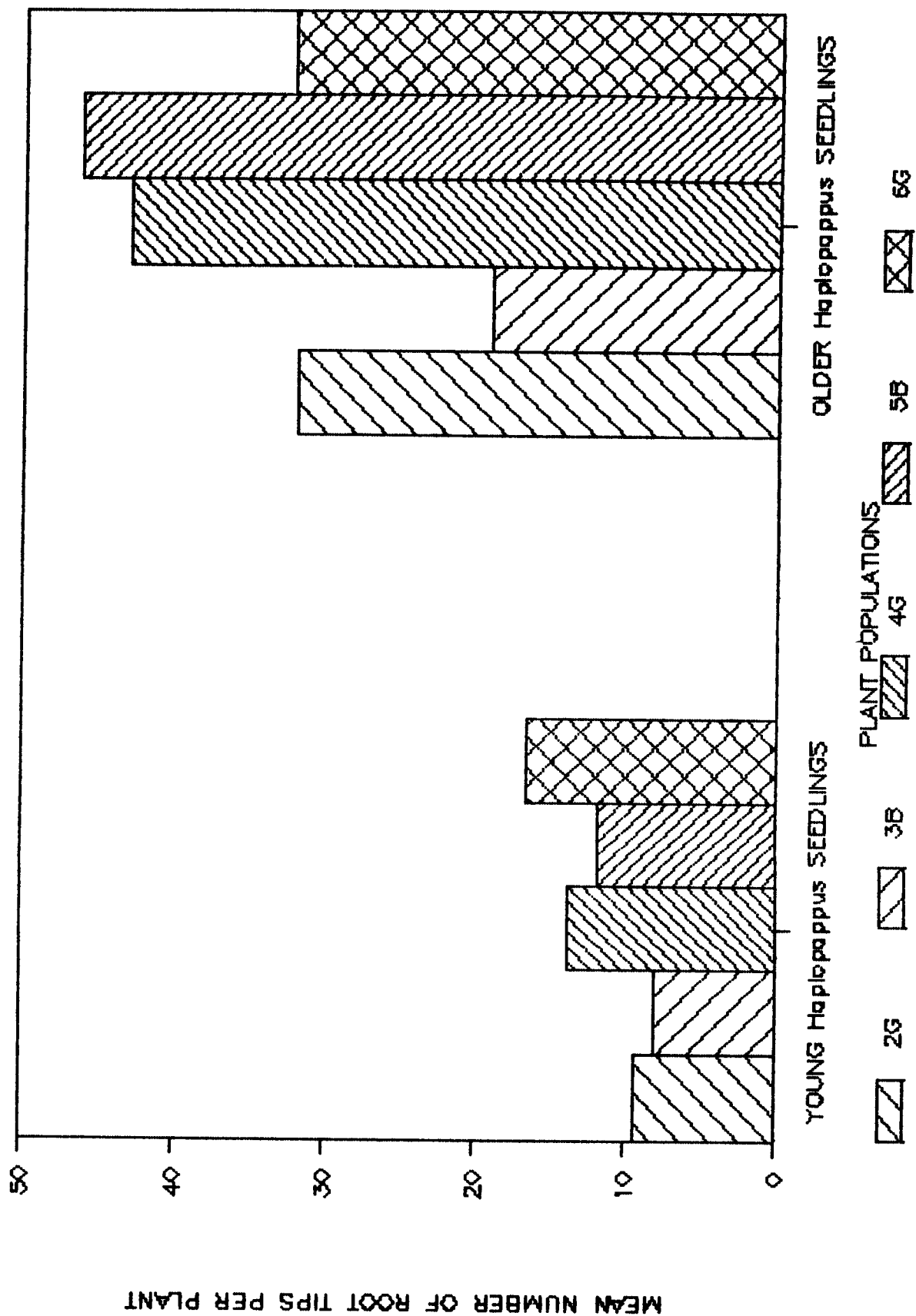
2G = PGC Position 2 - Green Foam

3B = PGC Position 3 - Brown Foam

4G = PGC Position 4 - Green Foam

5B = PGC Position 5 - Brown Foam

6G = PGC Position 6 - Green Foam



4. Haplopappus gracilis SEED STERILIZATION STUDIES

There is considerable interest in the comparison of tissue culture derived plantlets and their seed-derived counterparts. This is of particular importance to the CHROMEX project due to the phenomenon of somaclonal variation, in which tissue culture strains experience what are presumed to be increased rates of DNA alteration in culture. We have therefore initiated studies on the reliable procurement of sterile seedlings from Haplopappus gracilis. Technically, the germination units for Haplopappus are achenes, but for the sake of simplicity we shall refer to them as seeds throughout this report.

No single surface decontamination treatment will be always successful in producing axenic seedlings. Nevertheless, the goal is to establish a standard protocol which yields acceptable germination and sterility levels in a reliable fashion. The procedure chosen should be easily performed, inexpensive, safe, effective and with no deleterious effects on the plants (unless this is desired). Certainly there should be no mutagenic events stemming from the sterilization protocol. In this respect, some fungicides have been found to be mutagenic (Shields *et. al.*, 1984). In addition, antibiotics are commonly avoided in plant tissue cultures due to potential toxic effects. The practice of good sanitary procedures and the use of nonspecific sterilizing agents for surface treatment of tissues is emphasized (Schaffner, 1979).

The first question to be addressed concerned the normal degree of germination that can be expected in the absence of any potentially detrimental sterilization treatment. Four replicate treatments were set up with 20 seeds per container. This (as is the case in all subsequent seed sterilization studies) involved an initial selection of normal, healthy appearing seeds with a minimum size of ca 2 mm in length (excluding the pappus). Maximum seed lengths were slightly in excess of 3 mm, and the population's mode appeared to be around 2.5 mm. It is a long established practice to eliminate imperfectly formed seeds (Wilson, 1915; Duggar and Davis, 1919). In fact, the procurement of sterile seedlings often depends more on seed selection than on the decontaminating agent (Sweet and Bolton, 1979). In addition, a great deal of variability can be attributed to differences in degree of contamination between various batches of seeds.

Each of four glass jars contained a foam plug which was saturated with sterile distilled water. The seeds were inserted into the foam plugs (pappus end up) and scored daily for germination. The results are presented in Figure 17. After three days, germination rates for replicates 1, 2, 3 and 4 ranged from 30-75%. By day 10, however, the spread was down to 75-85% (mean = 80%), where it stabilized through day 16 and beyond.

Three major points come out of these data. First, in the absence of a harsh sterilization treatment, seeds from our KH-1 strain population (which were derived from plants grown in Japanese fields, harvested, and sent to us courtesy of Dr. R. Tanaka), germinate at a

Figure 17. Haplopappus gracilis Seed Sterilization Studies:

Percent Germination of Untreated Seeds

R1 = Replicate 1

R2 = Replicate 2

R3 = Replicate 3

R4 = Replicate 4

MEAN = Mean of Replicates 1-4

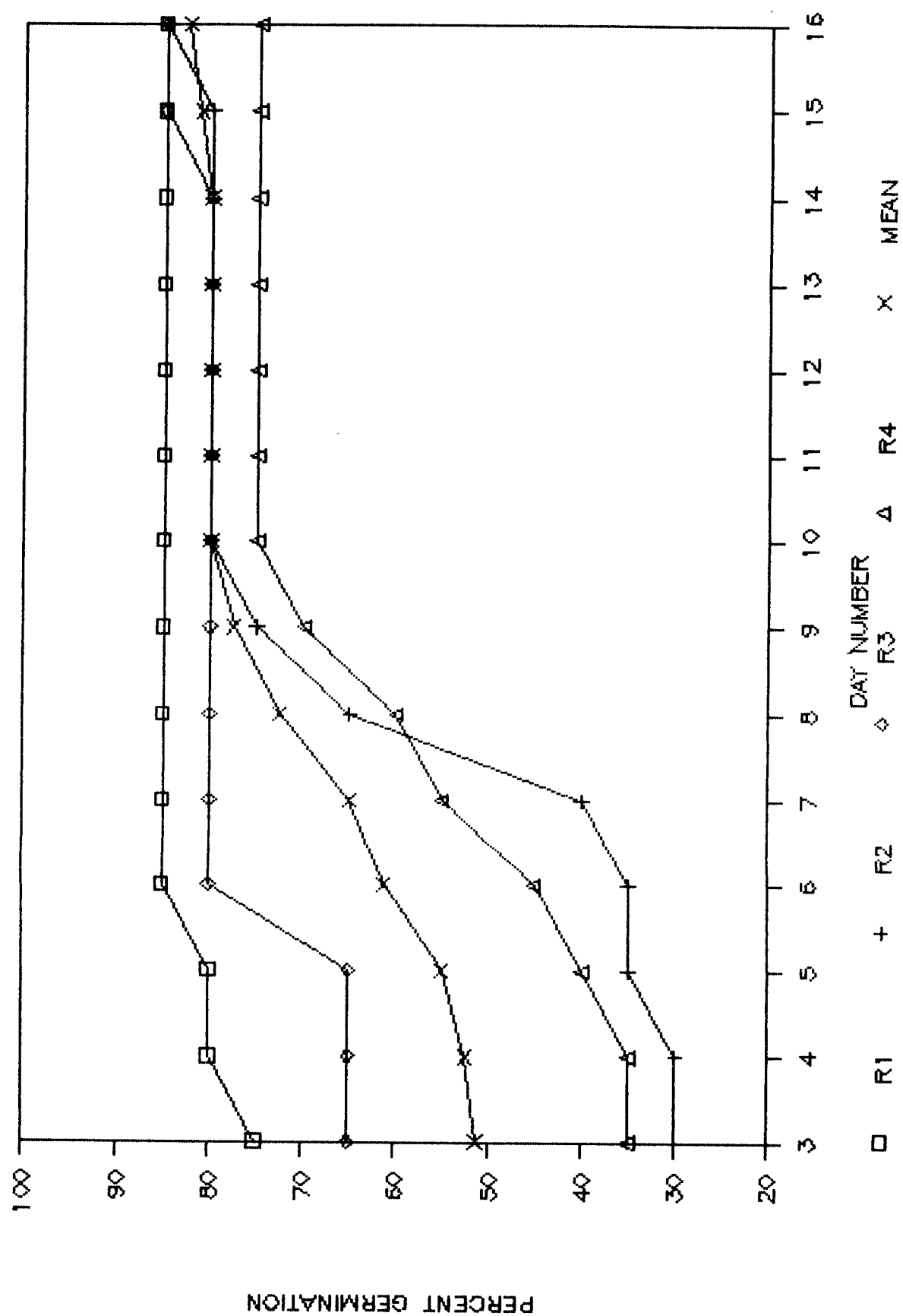


Figure 18. Haplopappus gracilis Seed Sterilization Studies:

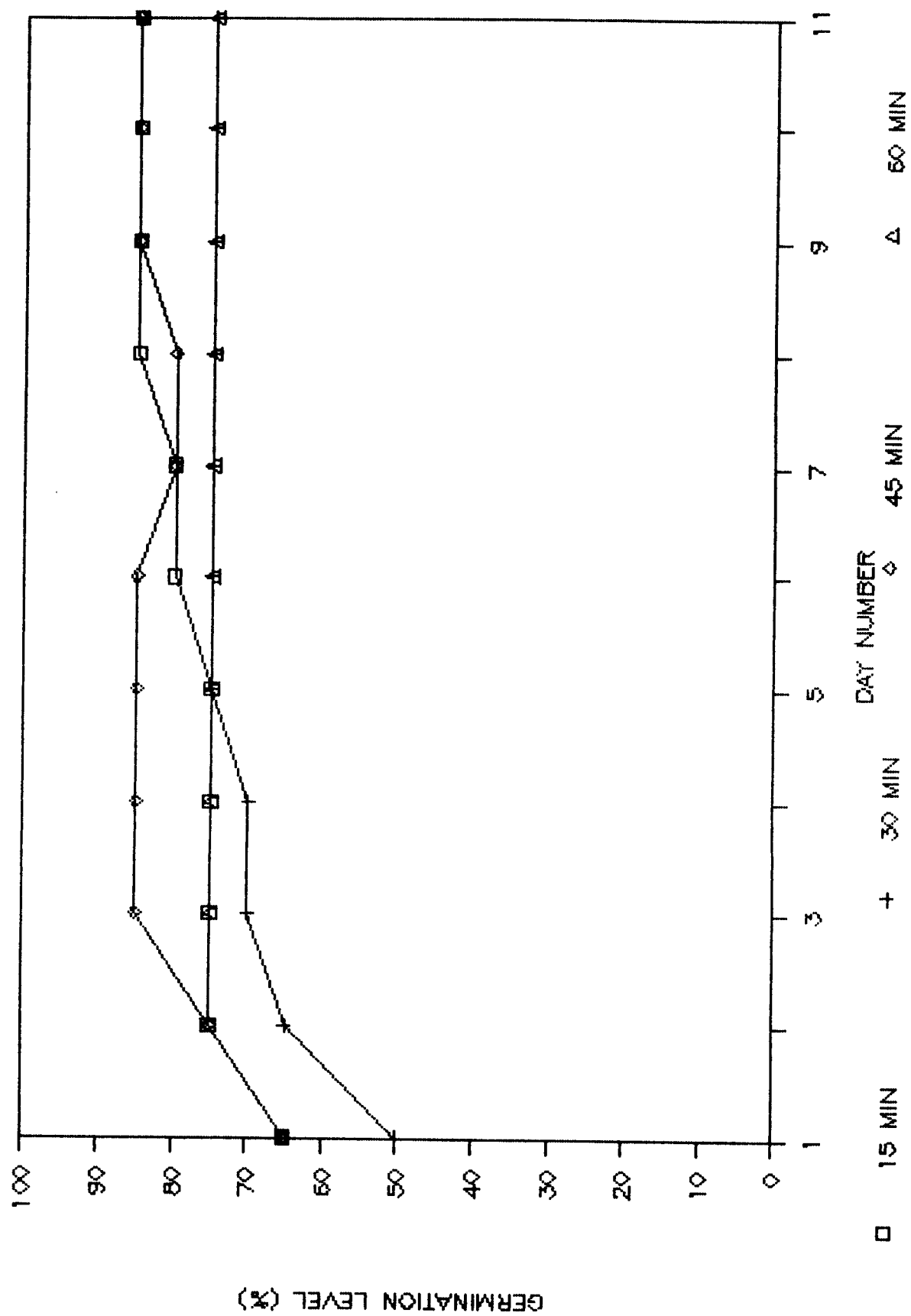
Percent Germination of Calcium Hypochlorite Treatments

15 Min = Seeds Treated for 15 Minutes

30 Min = Seeds Treated for 30 Minutes

45 Min = Seeds Treated for 45 Minutes

60 Min = Seeds Treated for 60 Minutes



rate of about 80%. Secondly, eleven days is generally long enough to achieve the eventual germination level. Sweet and Bolton (1979) also concluded that the incubation of seeds (in their case on microbial agar) should continue for at least eleven days (longer for slower germinating species). Third, the initial variability between replicates (certainly when the replicates consist of twenty or fewer seeds each) can be considerable. For this reason, it is not uncommon for seed investigations to employ 50 seeds per replicate (Dempsey and Walker, 1973).

Traditionally, we (and others) have primarily relied upon sodium hypochlorite in the form of the commercial product "Clorox" for the disinfection of seeds. Unfortunately, the potency of the product varies with age and storage conditions. In an attempt to settle upon a more reproducible protocol, attempts were undertaken to utilize calcium hypochlorite as the source of the active sterilizing ingredient, chlorine. It has been argued that the calcium salt is also preferable because sodium ions may adversely affect the growth of some seedlings (Sweet and Bolton, 1979).

Sweet and Bolton (1979) employed a 5% calcium hypochlorite solution (0.5% phosphate buffer, pH 6), which was subsequently diluted to 0.5% during their ten minute sterilization interval, followed by three sterile water rinses.

Several aspects of their procedures were attractive to us. Calcium hypochlorite was prepared as a 5% solution (active ingredient basis), filtered and stored in a tinted bottle in the refrigerator. It is known that acidifying to pH 6 greatly improves the effectiveness of hypochlorite as a source of active chlorine. The disinfecting solution was therefore maintained in a relatively inert state until immediately before use, at which point the pH was lowered to 6 and the solution began to rapidly lose chlorine gas. For the purpose of reducing the pH of the hypochlorite sterilization solution to 6-6.5, a solution was made containing 62.5 ml of concentrated HCl and 500 ml of 1 M KH_2PO_4 . They also employed a wetting agent stock solution since wetting agents reduce the contact times and concentrations required to achieve sterility. The wetting agent stock solution was a 1% (v/v) solution of either Triton or Tween 80 (both kept refrigerated). Thus, the sterilization solution was obtained by combining 174 ml sterile distilled water plus 20 ml 5% calcium hypochlorite solution plus 2 ml 1% wetting agent solution plus 4 ml of the buffer solution in an autoclaved beaker. The final hypochlorite level in the sterilization solution was a relatively low 0.5%.

We subjected Haplopappus seeds to the calcium hypochlorite solution (described above) for 15, 30, 45 and 60 minutes. The treated seeds were transferred to agar using forceps which had been flamed to incandescence and cooled. Seeds were planted one per bottle as recommended by Schenk and Hildebrandt (1972). The jars were incubated at 100-200 ft-c continuous fluorescent light at 22 C.

Daily observations were made on germination and other developmental processes. Figure 18 presents the percent germination data. All four treatments ended up with a 75-85% germination rate.

Figure 19. Haplopappus gracilis Seed Sterilization Studies:

Lateral Root Development of Calcium Hypochlorite Treatments

15 Min = Seeds Treated for 15 Minutes

30 Min = Seeds Treated for 30 Minutes

45 Min = Seeds Treated for 45 Minutes

60 Min = Seeds Treated for 60 Minutes

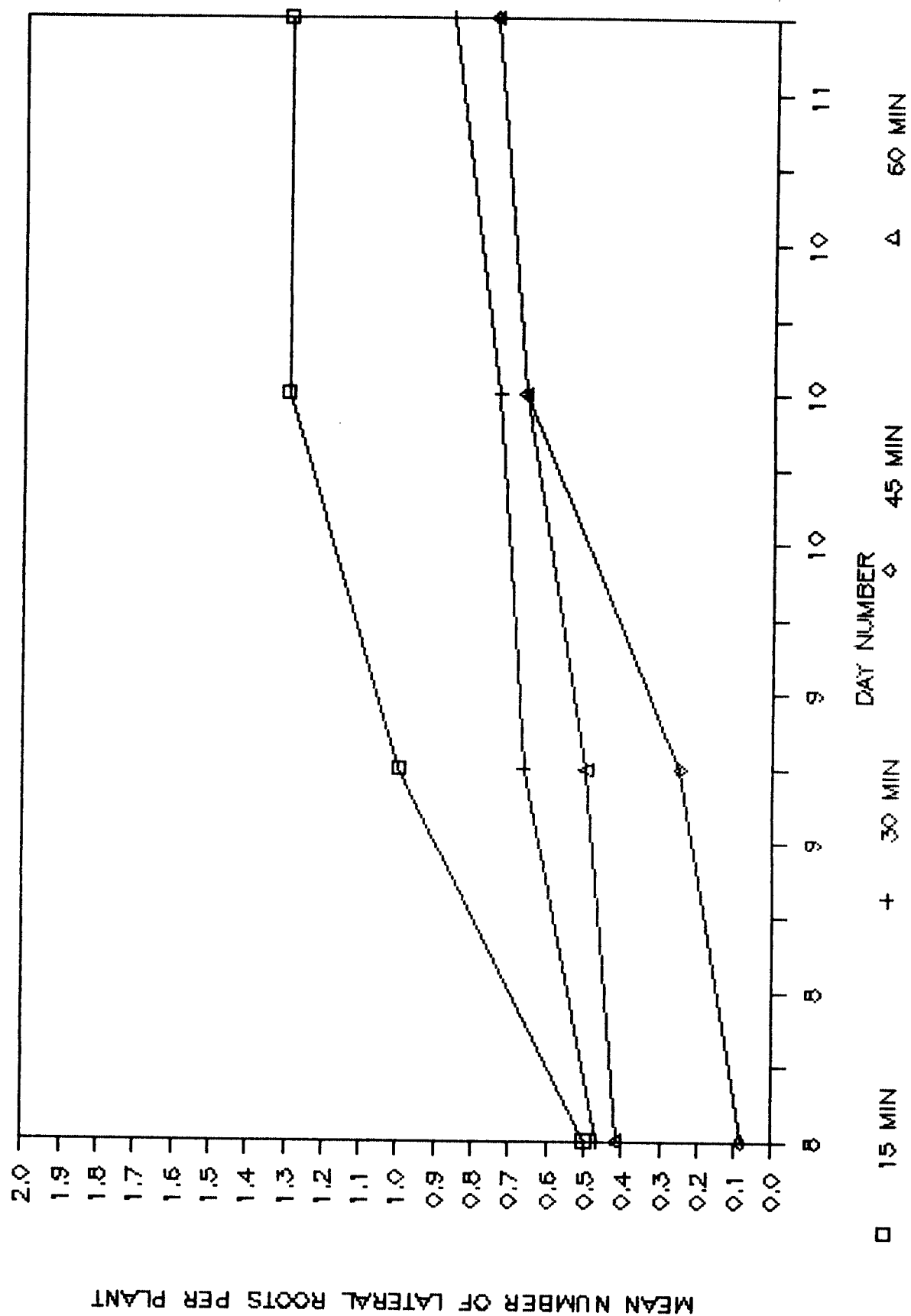


Figure 20. Haplopappus gracilis Seed Sterilization Studies:

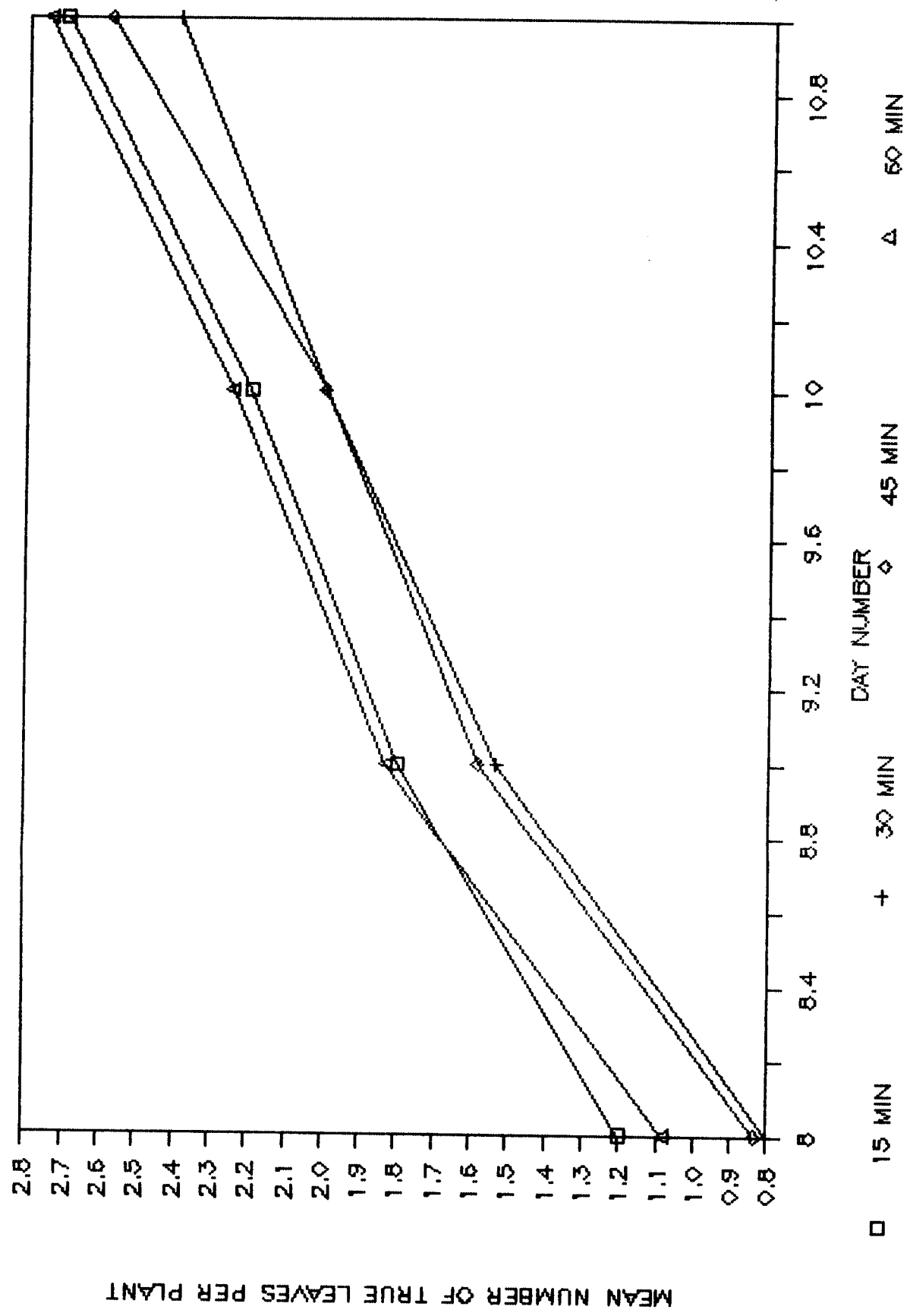
Leaf Development of Calcium Hypochlorite Treatment

15 Min = Seeds Treated for 15 Minutes

30 Min = Seeds Treated for 30 Minutes

45 Min = Seeds Treated for 45 Minutes

60 Min = Seeds Treated for 60 Minutes



Based upon the similar degree of germination exhibited by untreated seeds (Figure 17), we concluded that the calcium hypochlorite treatments were relatively benign. This can be attributed to the low concentration of active ingredient being used (0.5%). In contrast, "Clorox" or sodium hypochlorite treatments typically employ concentrations an order of magnitude higher. This compensates for solution deterioration. In addition, the technique of pH manipulation described above in the calcium hypochlorite protocol permits lower concentrations to work more effectively.

Having said that, closer examination of the seedlings obtained under the four calcium hypochlorite treatments revealed some subtle differences attributed to duration of exposure to the sterilization solution. The mean number of lateral roots branching off from seedling primary roots was found to decrease with increasing length of exposure (Figure 19). Given the importance of lateral root development in the CHROMEX project, the 15 minute exposure interval (or shorter) should be favored. This agrees with Sweet and Bolton (1979), who found that species with thin seed coats were often detrimentally affected by hypochlorite treatments of 10 minutes or longer.

We did not detect, however, decreased levels of true, non-cotyledonous, leaves with increasing exposure to the calcium hypochlorite sterilization solution (Figure 20). Other than the cotyledons, which can appear on the first day of germination, plants generally have one true leaf by day 8 and average about 2.5 leaves by day 11.

While the calcium hypochlorite treatments yielded excellent germination results, we frequently experienced a second wave of contamination which greatly reduced the number of usable plants. Some seeds have microbes which permeate the seed coat and beyond. In these instances, it may be necessary for the sterilizing agent to come into contact with the inner plant tissues, which can result in damage to the embryo (Sweet and Bolton, 1979). One precaution that can be taken is to remove the seed coats after germination, reducing contamination derived from organisms within the seed which can grow out over time (Sweet and Bolton, 1979). In addition, the pappus of Haplopappus species may serve as a source of contaminants.

These types of considerations led us to consider sulfuric acid sterilization treatments. This approach effectively burns off the outer seed coat as well as the pappus, reducing any contribution they may make to secondary waves of contamination.

Young (1919) reported obtaining excellent results when seeds were first dipped in 70% ethanol. The alcohol removes air cavities which would otherwise prevent proper contact between the disinfectant and the seed. Schaffner (1979) and Haldeman *et. al.* (1987), also employed 70% ethanol. We therefore decided to initiate the sterilization procedure with a .5-1.0 minute ethanol dip. Seeds were subsequently exposed to full strength sulfuric acid for 1, 2, 3 or 4 minutes, followed by four sterile distilled water rinses.

Figure 21. Haplopappus gracilis Seed Sterilization Studies:

Percent Germination of Seeds after Sulfuric Acid Treatments

1 Min = 1 Minute Exposure to Sulfuric Acid

2 Min = 2 Minute Exposure to Sulfuric Acid

3 Min = 3 Minute Exposure to Sulfuric Acid

4 Min = 4 Minute Exposure to Sulfuric Acid

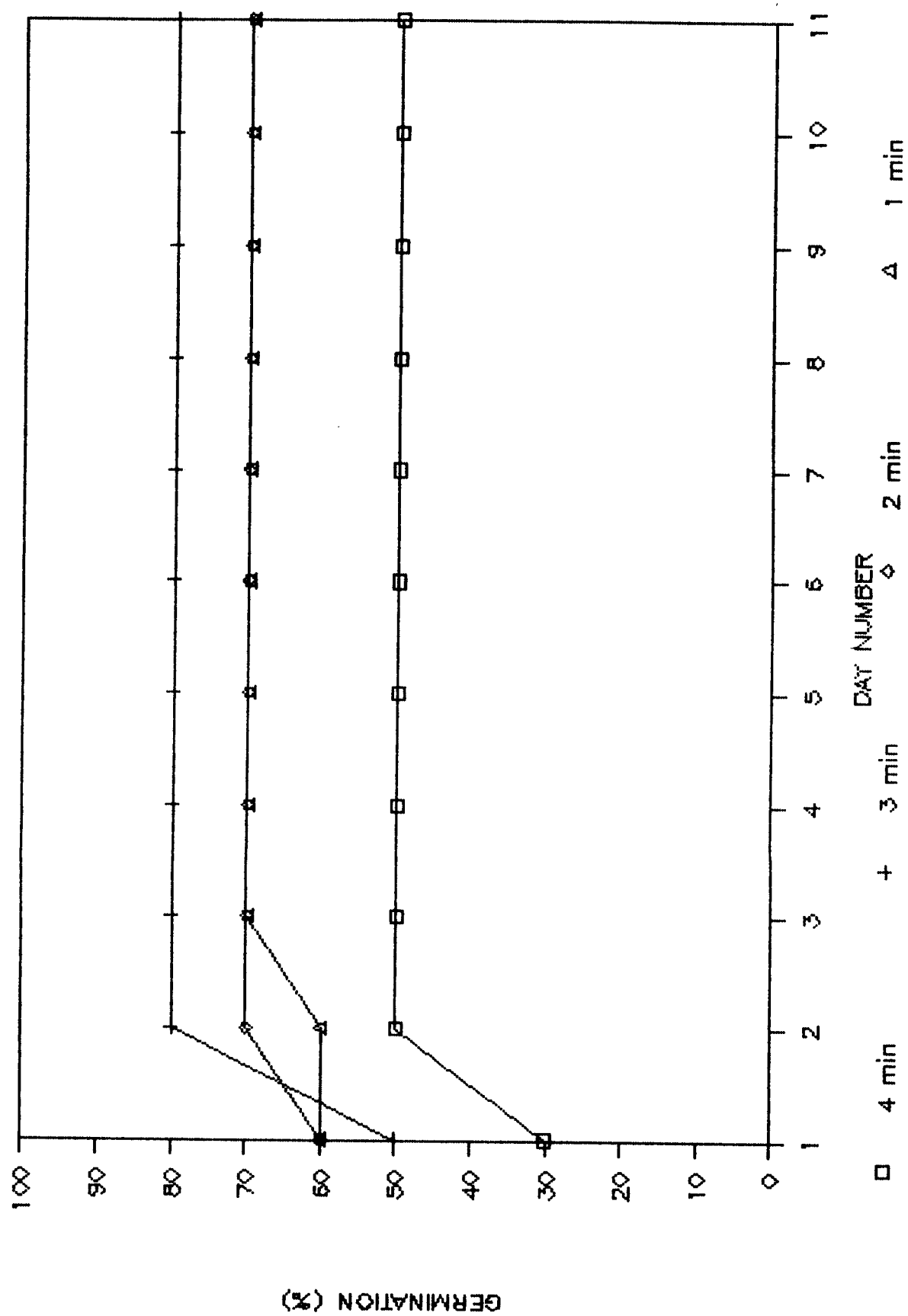


Figure 22. Haplopappus gracilis Seed Sterilization Studies:

Types of Germination Obtained after Sulfuric Acid Treatments

R = Normal Radicle-Led Germination

RA = Abnormal Radicle-Led Germination

C = Cotyledon-Led Germination

CR = Cotyledon/Radicle-Led Germination

0 = No Germination

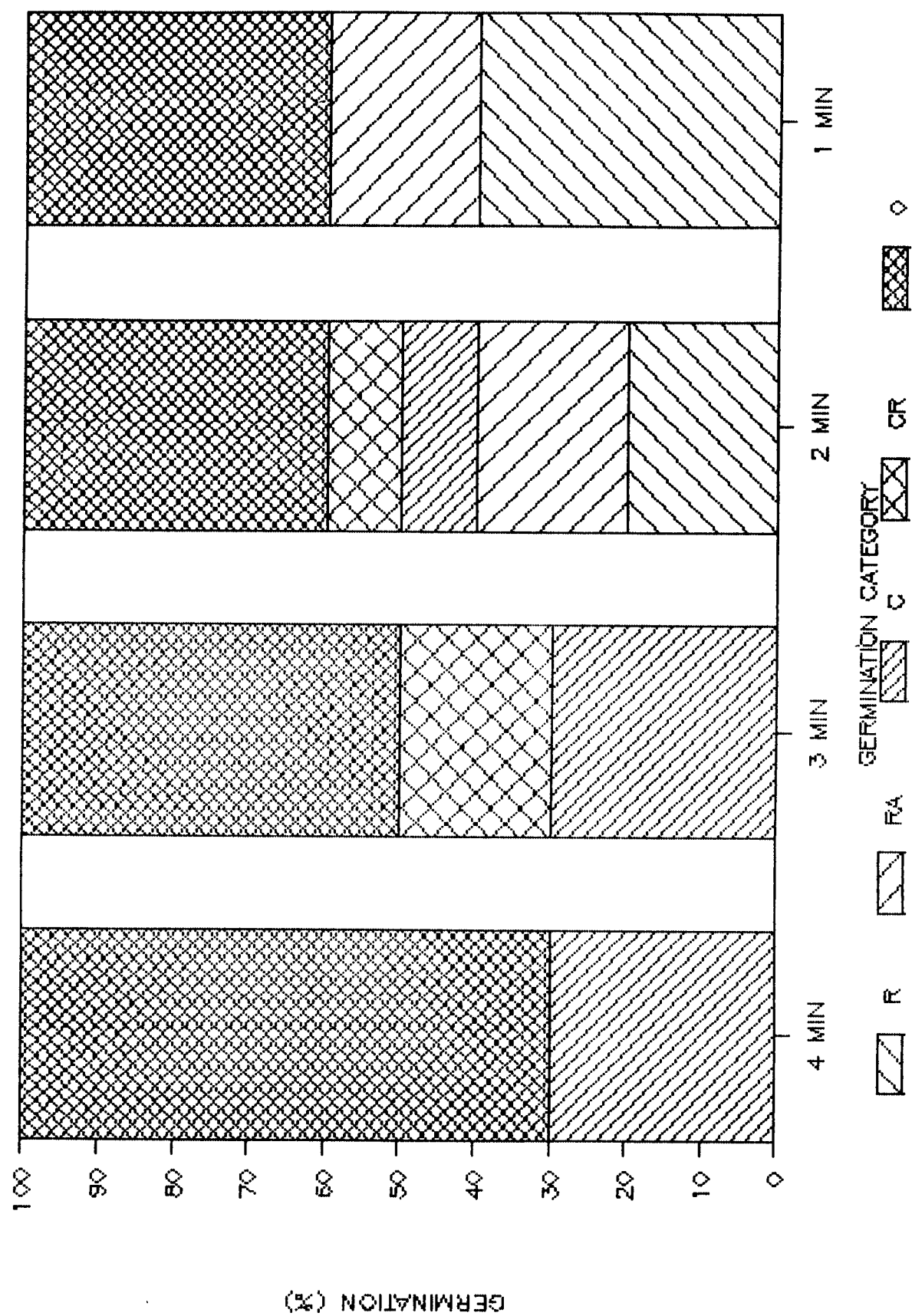


Figure 23. Haplopappus gracilis Seed Sterilization Studies:

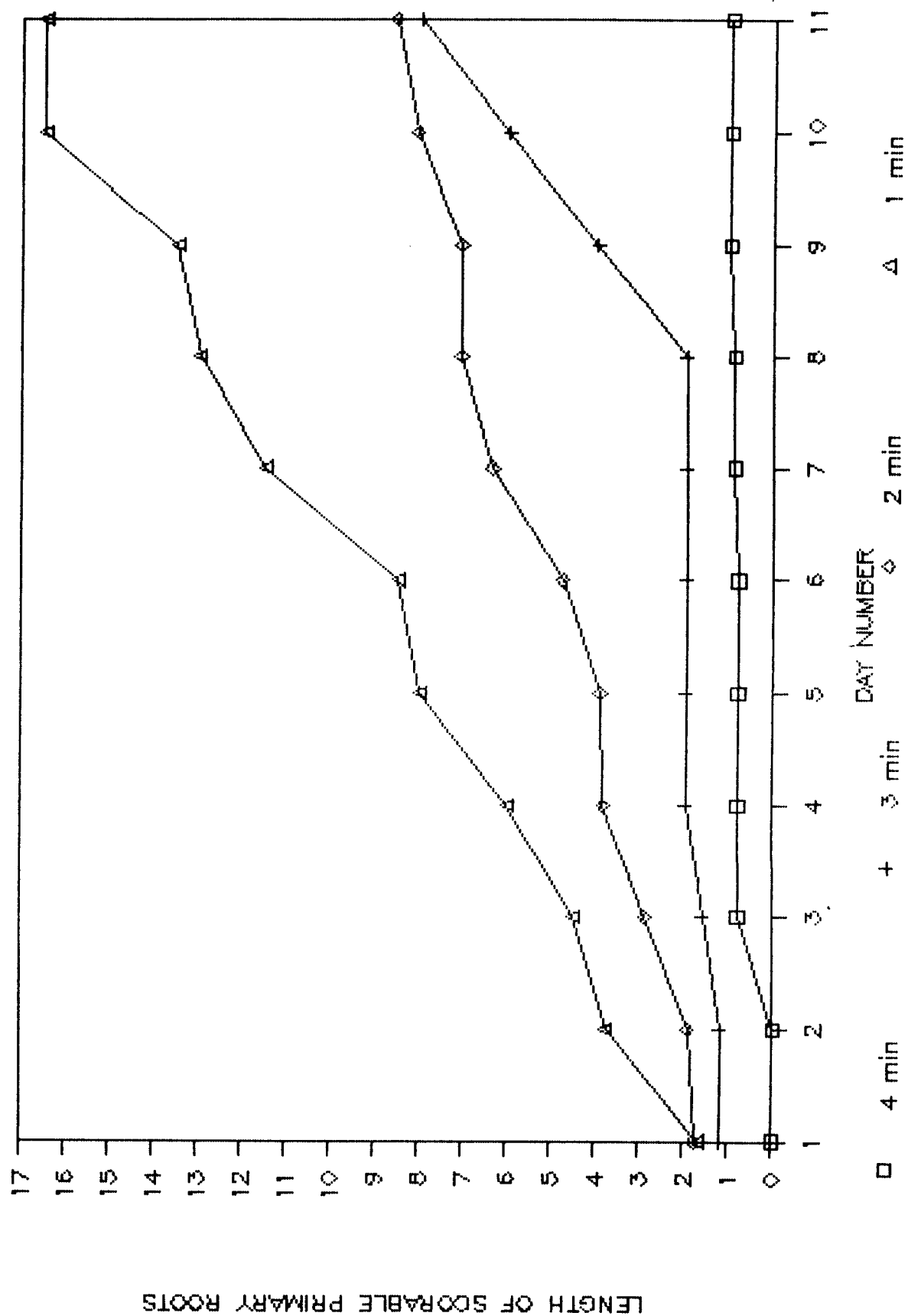
Primary Root Length Attained after Sulfuric Acid Treatments

1 Min = 1 Minute Exposure to Sulfuric Acid

2 Min = 2 Minute Exposure to Sulfuric Acid

3 Min = 3 Minute Exposure to Sulfuric Acid

4 Min = 4 Minute Exposure to Sulfuric Acid



Not surprisingly, the longest exposure (4 minutes) resulted in a depressed rate of germination (50%, Figure 21). In terms of percent germination, exposure durations of 1-3 minutes resulted in the comparatively normal level of 70-80% germination. These data are, however, somewhat deceptive when one considers certain abnormalities associated with seeds subjected to sulfuric acid treatments.

A normal Haplopappus gracilis seed begins germination with the radicle protruding through the tapered, ventral portion of the seed. As the radicle extends outward, the hypocotyl and cotyledons are pulled out through this ventral opening, eventually resulting in the entire embryo becoming disassociated from the seed coat. In contrast, we have observed what are being called "abnormal radicle-led germinations" in which the radicle begins to protrude outward, but then stops. This presumably results from damage inflicted upon the radicle root tip by the acid exposure. An even more severe abnormality is the so-called "cotyledon-led germination." In this case, acid damage prevents any extension of the radicle outward. However, the acid diminished the integrity of the cell wall to a sufficient degree that the cotyledons penetrate through midway up the seed wall. This usually resulted in a stunted germination. Another variation is the "cotyledon/radicle-led germination." In this instance, the cotyledons protrude outward through the seed wall in addition to some degree of radicle extension.

Figure 22 summarizes the occurrence of these germination types within the four sulfuric acid duration treatments. The highest degree of "normal germination" occurred with one minute acid exposures (slightly in excess of 50%). This dropped to about 40% with a two minute exposure and there were no "normal germinations" under the three and four minute treatments. Neither did the three and four minute treatments result in any of the "abnormal radicle-led germinations." Apparently, there was severe root tip damage in both of these exposure treatments. Instead, these treatments both had approximately 30% of their treated seeds undergoing the "cotyledon-led germination." Only about 10% of the two minute treated seeds germinated in this fashion, and none of the one minute treated seeds did so. Finally, 40% of the seeds failed to germinate at all in the one and two minute treatments. This rose to 50% under the three minute regime, and 70% for those seeds experiencing sulfuric acid for four minutes.

The results presented in Figure 23 reflect the degree to which the abnormalities described above affect the length of scorable primary roots. Based upon these data, the one minute sulfuric acid treatment is clearly favored.

We have successfully grown H. gracilis plants to maturity under both greenhouse and culture chamber conditions. Seeds planted in mid-November, 1987, produced plants in which flowers appeared during the first week of February. The first seeds were obtained in mid-March, resulting in a generation time on the order of 18 weeks (from seed to seed). In this instance, the harvested seeds had a low viability (due to a high degree of self-fertilization), but an extremely low degree of contamination. It is not uncommon to achieve zero contamination of

sterilized seeds from this batch. Germination rates in excess of 95% are obtainable if an adequate pre-selection of viable seeds is undertaken. As mentioned above, the primary factor affecting seed germination/contamination results is often the nature of the batch from which the seeds were derived. In this case, seeds grown within culture chambers which are not subjected to a prolonged exposure to moist conditions, contrast sharply with their field-grown counterparts in terms of fungal and bacterial infestations.

All of the above studies involved the germination of H. gracilis seeds in the absence of any plant growth regulators. At this time, we prefer the simplicity of this approach, and the lack of potential cytological complications. There are, however, reports of germination and DNA synthesis enhancement effects achieved through the use of gibberellic acid and fusicoccin (Galli et. al., 1975; Galli et. al., 1979). Future studies will assess the use of these compounds, and any implications they may have relative to rates of chromosomal aberrations.

CONCLUSIONS

- 1) Both the LC-1 (brown) and FFRC (green) foam formulations were found to be suitable for daylily and Haplopappus gracilis root growth studies. However, given the operating conditions of the PGU plant growth system, we currently favor use of the green FFRC foam due to its greater capacity for liquid retention.
- 2) The larger nitex mesh sizes were favored for root formation in daylily, and the smaller sizes were favored for H. gracilis. Roots have been observed to penetrate nitex mesh sizes 3 (and larger) for H. gracilis and nitex mesh sizes 6 (and larger) for daylily. Therefore, we currently favor the use of nitex sizes 1 and 2 for Haplopappus and size 5 for daylily.
- 3) We have consistently found there to be a gradient of increasing moisture content going downward through the foam blocks (for both formulations). This pattern is retained even as the blocks approach complete dryness, i.e., the uppermost portions, while always being dryer than lower levels, do not prematurely dry out. This pattern has also been documented for PGC-sized foam blocks subjected to a four day simulated shuttle flight at the Kennedy Space Center.
- 4) The number of exposed peripheral sides (of foam block subsections) is of much less significance than the downward percolation process (noted above) in describing the liquid distribution patterns within the foam blocks.
- 5) Sufficient root generation has been achieved under both laboratory conditions and within the EDU-PGC-AES plant growth apparatus to achieve the cytological objectives of CHROMEX. Even within the constraints of a 4-5 day shuttle mission, newly generated root tips have been procured. Efforts aimed at pushing the rate of root formation are continuing.
- 6) Daylily plantlets (tissue culture derived) grown in the presence of the antigibberellin ancymidol, have been found to produce more roots (and faster) than plantlets not subjected to ancymidol pretreatment.
- 7) When using the calcium hypochlorite seed sterilization protocol, an exposure duration of 15 minutes (or less) should be employed. Increased exposure durations reduced lateral root development.
- 8) When using the sulfuric acid seed sterilization protocol, an exposure duration of 1 minute (or less) should be employed. Longer durations result in unacceptably high rates of abnormal germinations, and drastically reduced the length of the newly formed roots.
- 9) We have successfully grown H. gracilis plants to maturity under both greenhouse and culture chamber conditions with a generation time of 18 weeks (from seed to seed). The harvested seeds had a low viability when self-fertilized, but a negligible degree of contamination when compared to their field-cultivated counterparts.

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